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Vol. 46, Nos. 3, 4 November 1956

PARASITOLOGY

(Founded by G. H. F. Nuttall)

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CONTENTS

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PAGE

WALKER, JANE B. *Rhipicephalus pravus* Dönitz, 1910. (With 11 Figures in the Text) 243

ARTHUR, DON R. The morphology of the British Prostriata, with particular reference to *Ixodes hexagonus* Leach. III. (With 69 Figures in the Text) . . . 261

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. I. Incidence in some birds of Hertfordshire. (With 2 Figures in the Text) 308

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. II. Transmission by *Ornithomyia avicularia* L. (With 1 Figure in the Text) 321

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. III. Life cycle in vertebrate and invertebrate hosts. (With 5 Figures in the Text) . . . 335

THEODOR, OSKAR. On the genus *Tripselia* and the group of *Basilia bathybothyra* (Nycteribiidae, Diptera). (With 57 Figures in the Text) 353

RAUSCH, ROBERT and SCHILLER, EVERETT L. Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of *Echinococcus sibiricensis* Rausch & Schiller, 1954, on St Lawrence Island. (With Plates VII and VIII, and 3 Figures in the Text) 395

KULASIRI, C. and FERNANDO, C. H. Camallanidae parasitic in some Ceylon fish . 420

REES, GWENDOLEN. The scolex of *Tetrabothrius affinis* (Lönnberg), a cestode from *Balaenoptera musculus* L., the blue whale. (With 24 Figures in the Text) . . 425

NAJIM, ABBAS T. Life history of *Gigantobilharzia huronensis* Najim, 1950. A dermatitis-producing bird blood-fluke (Trematoda-Schistosomatidae). (With 19 Figures in the Text) 443

BEARUP, A. J. Life cycle of *Austrobilharzia terrigalensis* Johnston, 1917. (With 9 Figures in the Text) 470

GOODRICH, HELEN PIXELL. Crayfish epidemics 480

Index of Authors 484

Index of Subjects 486

5847

Printed in Great Britain at the University Press, Cambridge
(*Brooke Crutchley, University Printer*)

PARASITOLOGY is published in double issues twice a year. The numbers afterwards are issued in volumes each containing four numbers.

Papers for publication should be sent to Professor D. KEILIN, Molteno Institute, Downing Street, Cambridge. Other communications should be addressed to the University Press, Cambridge.

Papers forwarded to the Editors for publication are understood to be offered to *PARASITOLOGY* alone, unless the contrary is stated.

Contributors receive fifty copies of their papers free. Additional copies, not exceeding one hundred (except in special cases), may be had at cost price: these should be ordered when the final proof is returned.

The subscription price, payable in advance, is £4. 10*s*. net per volume, post-free (U.S.A. $15.00), double numbers 45*s*. net, postage extra (U.S.A. $8.50). Subscriptions may be sent to any Bookseller, or to The Cambridge University Press, Bentley House, 200 Euston Road, N.W. 1.

Enquiries from the U.S.A. should be addressed to the American Branch of the Press at 32 East 57th Street, New York, 22.

RHIPICEPHALUS PRAVUS DÖNITZ, 1910

BY JANE B. WALKER

East African Veterinary Research Organization, Muguga, P.O. Box 32, Kikuyu, Kenya Colony

(With 11 Figures in the Text)

INTRODUCTION

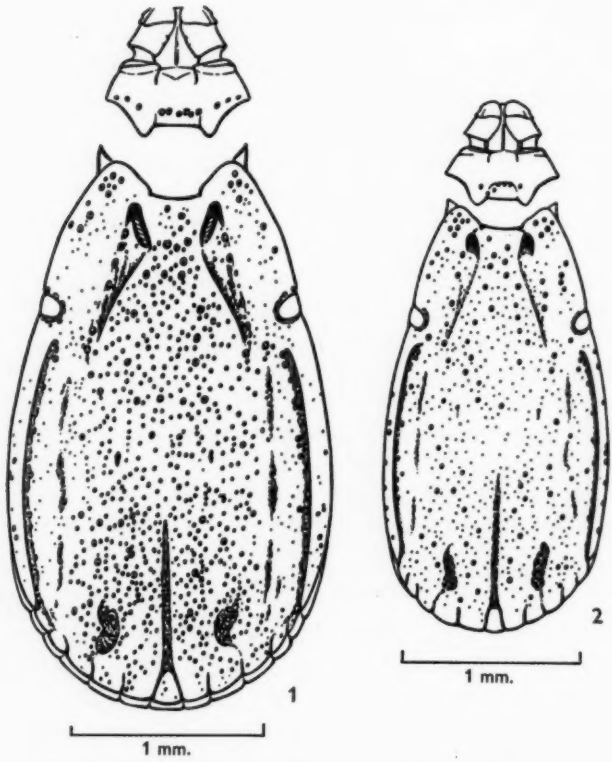
The adults of *Rhipicephalus pravus* were first described very briefly by Dönitz, in 1910, from specimens collected from buffalo, giraffe and various antelopes on the Masai Steppe in Tanganyika. He did not designate any specific specimens as types. He compared the species with *R. oculatus*, but stated that the difference in the structure of the eyes of the two forms, which he described in detail, was so marked that he considered it to be specific. He also commented on the fact that the males of *R. pravus* are often strikingly narrow anteriorly, but added that this is not a decisive character. Zumpt (1942) redescribed the adults in detail from material collected in the Tanganyika highlands (Arusha, Kilimatinde, Kondoa-Irangi, Mkalama, Morogoro and Mpwapwa), in Kenya (Mt. Nyro and Mt. Loroghi) and in Abyssinia (Diredawa). Theiler & Robinson (1953) gave descriptions of adults collected at Fairmount, Edenburg, Orange Free State and of nymphae and larvae bred from them in the laboratory. They also discussed the biology, distribution and synonymy of the species. The following descriptions of the adults, nymph and larva are based on laboratory-bred specimens, the progeny of a female collected at Kima, approximately 90 miles south-east of Nairobi.

DESCRIPTION

Male (Figs. 1–4)

Conscutum. Size 3 × 1·8–1·9 × 1·2 mm. Inornate, reddish brown, often narrow anteriorly and elongate. Subcollare and anterior process of coxa I well developed. Emargination wide and deep. Eyes usually rather convex, oval in shape and set obliquely, dorsally edged by punctations which may coalesce and form a groove, ventrally flush with surface. Cervical pits deep and convergent; cervical grooves convergent at first then becoming divergent and fading out beyond level of eyes, usually fairly well marked, but in some cases merely represented by fine superficial pitting; lateral grooves represented by somewhat irregular rows of medium-sized punctations. Cervical fields very characteristic in shape, being relatively broad anteriorly and tapering to points posteriorly beyond the level of the eyes, but sometimes rather indistinct in outline. Marginal grooves always well developed, sometimes almost including 1st festoon, outer edges sharply defined, inner edges irregular and picked out by punctations. Superficial pitted depressions present in position of dorsal foveae and along two roughly parallel lines inside marginal grooves. Postero-median and postero-lateral grooves all well

developed with rugose surfaces, usually continuous with adjacent intervals of festoons; postero-median groove long and narrow, postero-laterals short and broad. Festoons short, intervals broad; outer festoons often smaller than the rest and irregular in shape. Caudal appendage with terminal disc present, arising from central festoon. Punctation pattern very variable, made up of discrete medium-sized and small punctations. Typically medium-sized punctations are grouped on the scapulae, in lines along the positions of the lateral grooves, round



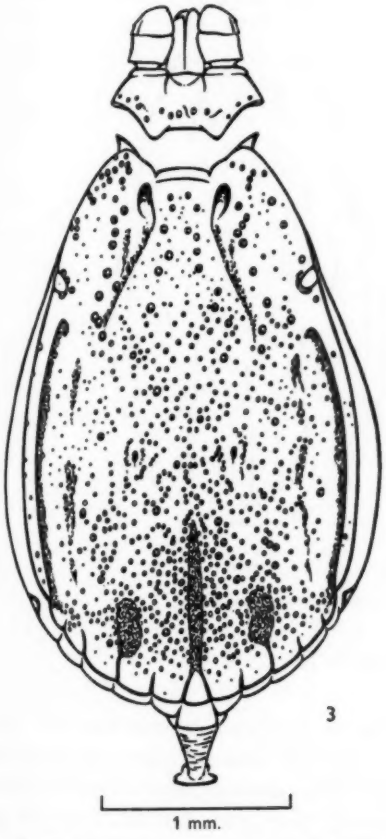
Figs. 1, 2. *R. pravus*, laboratory-bred males.

the dorsal edges of the eyes and scattered over the rest of the surface of the conscutum, except in the areas just inside the marginal grooves. Smaller punctations are present all over the conscutum, those round the eyes and in the areas inside the marginal grooves being particularly fine; the latter sometimes almost disappear. Figs. 1 and 2 represent males from the same laboratory-bred series showing the extremes in the development of the punctation pattern. In one the small punctations are relatively prominent and dense, in the other they are fine and superficial. Fig. 3 represents a male from the British Museum collection, presented by Dönitz to Nuttall.

Legs. Increase in size from I to IV.

Capitulum. Slightly broader than long excluding the cornua.

Basis capituli. Twice as broad as long excluding the cornua, lateral angles prominent and curved towards ventral surface, cornua well developed. A row of medium-sized punctations present roughly parallel to posterior edge, which is nearly always straight but may be slightly concave. In very small specimens the basis capituli tends to be relatively narrower and the recurved lateral angles appear to be more pronounced.



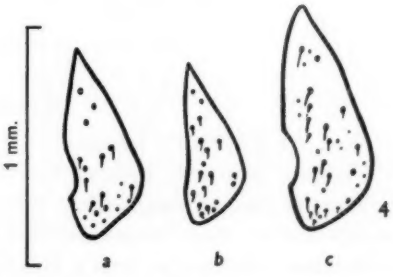


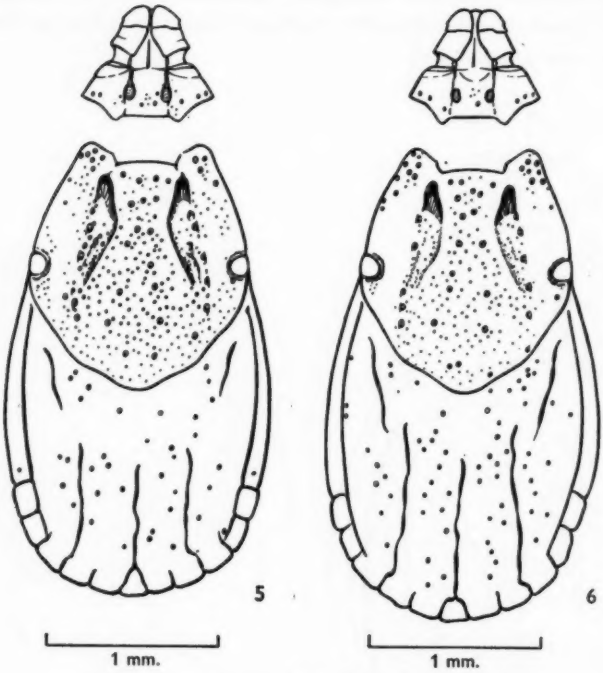
Fig. 3. *R. pravus*, male from British Museum collection presented by Dönitz.

Fig. 4. *R. pravus*, anal plates of males: *a*, *b*, laboratory-bred specimens; *c*, specimen from British Museum collection presented by Dönitz.

Palps. Broad and short. Article I easily visible from dorsal surface. Article II longer and somewhat broader than article III, which shows a small tooth-like externo-lateral projection.

Ventral surface. Anal plates (Figs. 4*a–c*). Vary considerably in shape. Internal margin usually scooped out opposite anus, antero-internal angle often forming a

small, heavily chitinized point, postero-internal angle sometimes almost a right angle, sometimes more acute; external margin slightly sinuous to straight, meeting the posterior margin in a broad curve. Surface heavily punctate. *Accessory anal plates.* When present are represented by small chitinized points but are sometimes absent altogether, especially in very small specimens.



Figs. 5, 6. *R. pravus*, laboratory-bred females.

Female (Figs. 5–7)

Size. 3 × 1·6–2·4 × 1·3 mm.

Scutum. Inornate, reddish brown, elongate, posterior border sinuous. Subcollare present. Emargination wide but shallower than in male. Eyes usually rather convex, more subcircular than oval in shape, dorsally edged by punctations which may coalesce to form a groove, ventrally flush with surface. Cervical pits deep and convergent, continuous with cervical grooves, which are convergent at first then become divergent and fade out beyond level of eyes; as in male they are usually fairly well marked, but in some cases are merely represented by fine superficial pitting. Lateral grooves represented by rows of medium-sized punctations which tend to run almost parallel, thus accentuating the elongated appearance of the scutum. Cervical fields very characteristic in shape, being relatively broad anteriorly and tapering to points posteriorly beyond the level of the eyes. Punctation pattern very variable, made up of discrete medium-sized and small punctations. Medium-sized punctations are grouped on the scapulae, in rows along

the positions of the lateral grooves, round the dorsal edges of the eyes and scattered over the median portion of the scutum. Smaller punctations may be scattered all over the scutum; when present those on the raised lateral borders and round the eyes are particularly fine, in some cases they are completely absent in these areas, which are then smooth and shiny. Figs. 5 and 6 represent females from the same laboratory-bred series showing the extremes in the development of the punctation pattern. Fig. 7 represents a female from the British Museum collection, presented by Dönitz to Nuttall.

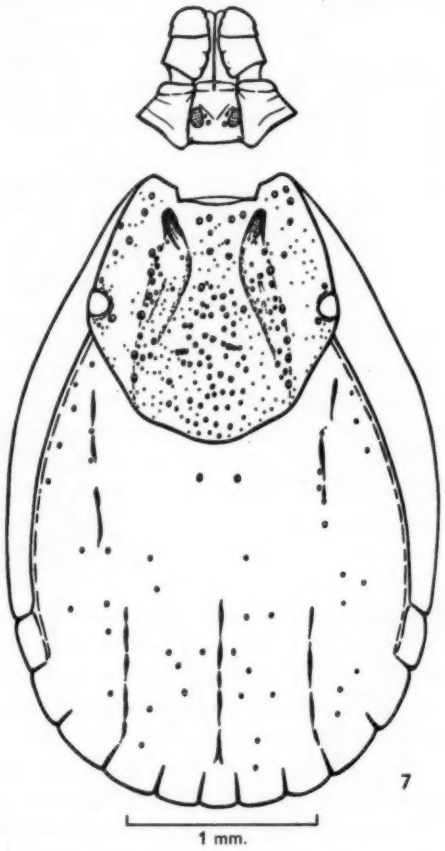


Fig. 7. *R. pravus*, female from British Museum collection, presented by Dönitz.

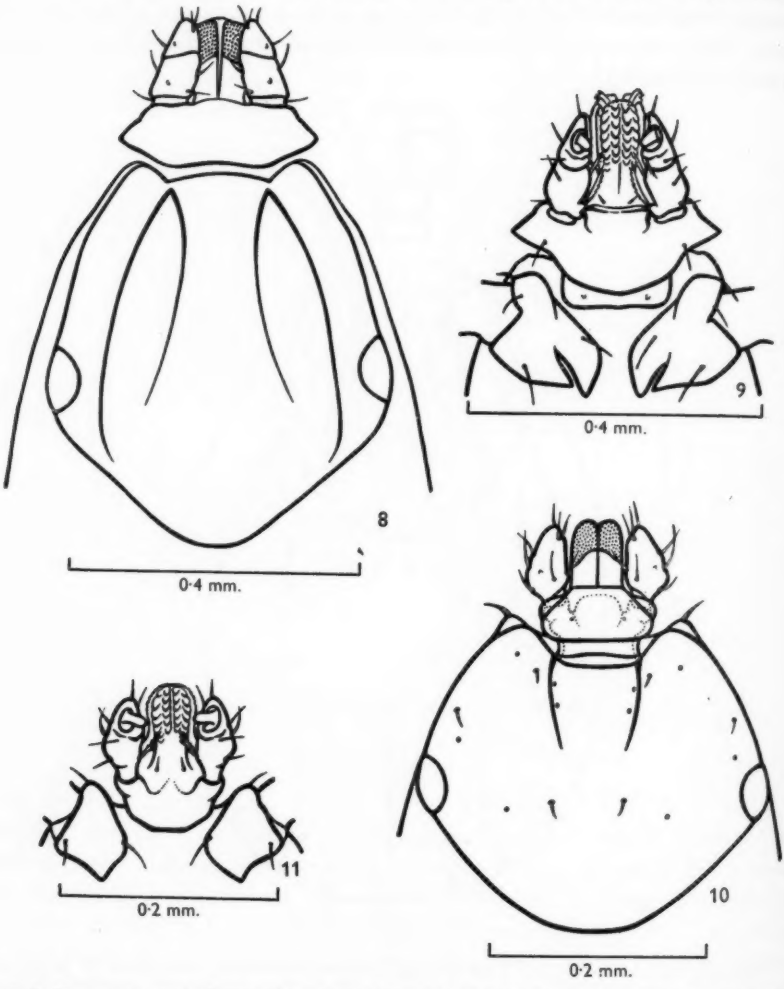
Capitulum. Slightly broader than long.

Basis capituli. Twice as broad as long; lateral angles prominent and somewhat curved towards ventral surface, cornua well developed. Median portion, in which the porose areas lie, sunken. Porose areas variable in form, kidney-shaped to oval, relatively large to small in size. Surface of basis capituli punctate.

Palps. Broad and short. Article II longer and slightly broader than article III. Tip of article III bluntly rounded.

Nymph (Figs. 8, 9)

Scutum. Longer than broad, posterior border sinuous, smoothly rounded at end. Emargination wide and deep. Eyes large, just in front of widest part of scutum. Cervical pits deep; cervical grooves deep at first, becoming more and



Figs. 8, 9. *R. pravus*, capitulum and scutum of nymph. Fig. 8, dorsal view; Fig. 9, ventral view. Figs. 10, 11. Capitulum and scutum of larva. Fig. 10, dorsal view; Fig. 11, ventral view.

more shallow and fading out at about the level of the eyes. Lateral grooves much more sharply defined, reaching almost to posterior border of scutum and then curving inwards. They cut off well-marked lateral borders and have the effect, as seen in the female, of accentuating the elongated appearance of the scutum.

Capitulum. Broader than long.

Basis capituli. Three times as broad as long, smooth looking. Lateral angles blunt; antero-lateral borders straight, postero-laterals and posterior border straight to slightly concave. No cornua.

Palps. Normally slope towards one another and their tips almost touch. Article II longer and slightly broader than article III, which tapers to a smoothly rounded end.

Larva (Figs. 10, 11)

Scutum. Broader than long, posterior border evenly curved. Eyes approximately half-way back. Cervical pits continuous with short convergent cervical grooves.

Capitulum. About as broad as long.

Basis capituli. Twice as broad as long, slightly broader anteriorly than posteriorly with no lateral angles or cornua. Lateral margins continue through smooth curves into the straight posterior margin.

Palps. Broad at the base and tapering toward the tips, which are rounded. Articles II and III not visibly separated into two units.

DISCUSSION

The series of ticks, bred in the laboratory, on which the above descriptions have been based were initially identified as *R. neavei*. They show a considerable range of variation in their structure, this variation being particularly noticeable in the punctation patterns. Examination of specimens of *R. pravus* (Figs. 3, 7) in the British Museum collection shows that they fall within the range of variation shown by our laboratory-bred series. These specimens are from a batch 'off Oryx Antelope from the Southern Borders of the Masai Steppe, German East Africa' presented by Dönitz to Nuttall in 1911, and it is probably the batch referred to by Warburton (1912) in the footnote to his description of *R. neavei punctatus*. Thus *R. neavei* falls as a synonym of *R. pravus*. Theiler & Robinson (1953), comparing the paratypes of *R. pravus* with relatively heavily punctate ticks initially identified as *R. neavei punctatus*, came to the conclusion that the latter is a synonym of *R. pravus*. It would appear, therefore, that our more lightly punctate specimens, corresponding to *R. neavei*, represent one end of the possible range of variation of *R. pravus* and that more heavily punctate specimens, corresponding to *R. neavei punctatus*, represent the other end. Nuttall (1913) has already shown that there is a similar range of variation in the structure of *R. appendiculatus*, and Cunliffe (1914) in that of *R. sanguineus*.

A comparison of some of the type specimens of *R. neavei* and *R. neavei punctatus* in the British Museum collection (which now includes the Nuttall collection from Cambridge as well as the Entomological Research Committee collection) with Dönitz's specimens of *R. pravus* shows these to be synonyms of *R. pravus*. The types of *R. neavei* from both collections, from an eland near the mouth of the Tasangazi River, Luangwe Valley, north-east Rhodesia, collected by S. A. Neave on 1 September 1910, show considerable variation in the development of the superficial pitted depressions seen in the position of the foveae and just inside

the marginal grooves in the males as well as in the punctation patterns. (In Warburton (1912) the spelling of the river's name is given as 'Tasangazi', on the label with the type specimens in the British Museum collection it is given as 'Lasangazi' and on maps of the area it appears as 'Lusangazi'.) In some specimens the punctations are quite dense and deep, in others more sparse and superficial. The eyes are relatively prominent, dorsally edged with punctations and ventrally flush with the conscutum. In the case of *R. neavei punctatus* only the types from the Nuttall collection, no. 1411, two males and one female collected from a kudu near Fort Mlangeni, Central Angoniland, by S. A. Neave in May 1910, were examined. In one male the cervical fields are not so pronounced as usual, but all the specimens are very heavily punctate and the punctations tend to be uniform in size. This tendency towards uniformity is due to the fact that the interstitial punctations, which are usually relatively small and superficial, are larger and deeper than they are in many specimens of *R. pravus*, but they still do not quite equal the larger punctations in size. In all three ticks the eyes are somewhat bulging and edged by punctations dorsally.

A careful comparison of the descriptions and illustrations of *R. pravus* given above, with those given by Dönitz, Zumpt, and Theiler & Robinson and with Warburton's descriptions of *R. neavei* and *R. neavei punctatus*, does not reveal any significant differences between them. Some of the differences between the various illustrations are probably due to the fact that the ticks from which they were drawn were lying at different angles, e.g. the basis capituli of the nymph illustrated by Theiler & Robinson appears to be tilted upwards more than that illustrated by the author. Theiler (private communication) has also stated that in their figures of the nymph the eyes are a little too far forward on the scutum and the lateral angles of the basis capituli are too blunt.

It would seem that Warburton himself later also decided that *R. neavei* and *R. neavei punctatus* would fall within the range of *R. pravus*. In 1932 he identified a very variable batch of 'males and females off *Oreotragus* male shot at Ishingiro Hill, 5200 ft., Ankole, Uganda, 2. xii. 1931, received from W. F. Poulton—Nuttall Collection no. 3895A' as *R. pravus*. One male in this series is very lightly punctate, the interstitial punctations being reduced almost to pin points, and it corresponds to his *R. neavei*, but most of the remaining sixteen males and ten females tend to be rather heavily punctate and would correspond more to *R. neavei punctatus*. Another batch, Nuttall Collection no. 2385b, has been labelled, presumably by Warburton, as '*Rhipicephalus neavei* Warb., females off eland, no data, from R. Hancock (=*R. pravus* Dö.)'.

Both *R. neavei* and *R. neavei punctatus* (ex parte) were sunk as synonyms of *R. appendiculatus* by Zumpt (1942). In 1949 he reinstated *R. neavei* but still gave *R. neavei punctatus* (part ?) as a synonym of *R. appendiculatus*. Dias (1951) redescribed *R. neavei punctatus* and gave it specific status as *R. punctatus*. This, therefore, also falls as a synonym of *R. pravus*.

R. appendiculatus can be distinguished from *R. pravus* by a number of features. The basis capituli of the male is not usually so broad as it is in *R. pravus* and the obtuse lateral angles are more anterior in position. The conscutum widens markedly

behind the eyes, being widest about half-way between the eyes and the posterior edge of the conscutum. The eyes are fairly small and almost flush with the conscutum. The cervical fields are very characteristic in shape and much broader than those of *R. pravus*, with surfaces which are finely and evenly pitted. There are no superficial pitted depressions parallel with the marginal grooves, the two areas inside these grooves are smooth and practically devoid of punctations. The anal plates are relatively narrow and elongated and the posterior angles are smoothly curved rather than bluntly right-angled as in *R. pravus*. In the *R. appendiculatus* female the eyes, as in the male, are not so large or prominent as they are in the *R. pravus* female, and the cervical fields are large and broad. *R. appendiculatus* is not as a rule found in the same areas as *R. pravus* as it appears to be unable to survive under such dry conditions as does the latter.

In their discussion on its systematics, Theiler & Robinson (1953) state that 'it is Zumpt's opinion that *R. pravus* has been listed as *R. oculatus* by several of the older workers, including Neumann' but, after working through the records and studying some of Lewis's material, they consider that it is more likely to have been confounded with *R. bursa*. That it has also been confused with *R. oculatus* is shown by the presence of specimens in the British Museum collection labelled '*Rhipicephalus oculatus* (*pravus form*)'. The structure of the eyes of *R. oculatus*, which are small, beady and orbited, resembling those of *R. evertsi* and unlike those of *R. pravus*, which are larger, more oval in shape and not completely orbited, should serve to distinguish these two species.

Present-day findings suggest that *R. bursa* is not found in Africa south of the Sahara. It is a larger and somewhat broader tick than *R. pravus*. The capitulum of the male is more compact and the lateral angles of the basis capituli, although acute, are more anterior in position. The eyes are relatively small and the cervical fields, though long and narrow, are not very definitely outlined. No superficial pitted depressions on the conscutum, parallel with the marginal grooves, are seen, but there may be a number of rather irregularly shaped hollows, especially just anterior to the postero-lateral grooves. The anal plates are large and much broader posteriorly than those of *R. pravus*. In the female of *R. bursa*, as in the male, the eyes are relatively small and not very prominent. The cervical fields are much broader than those of the *R. pravus* female and reach to the posterior border of the scutum, and the lateral borders of the scutum, in front of the eyes, are punctate and not so smooth as they are in *R. pravus*. Theiler & Robinson (1953) comment that as early as 1905 Dönitz suggested that 'the older records of the occurrence of *R. bursa* in South Africa might not have been reliable and that hence many incorrect identifications were made'. They consider that many of the records of *R. bursa* in Africa south of the Sahara given by Neumann (1897, 1901) and Howard (1908) probably represent records of *R. pravus* and not *R. oculatus* as surmised by Zumpt. Theiler & Robinson (1954 and private communication correcting misprint in this paper) list as *R. pravus* the ticks from the Congo recorded as *R. bursa* by Newstead, Dutton & Todd (1907), Schwetz (1927) and Bequaert (1931). These records will be included in the section in this paper dealing with the distribution of this species, although none of the ticks have been seen either by

the author or by Theiler & Robinson, and our surmise that they do in fact represent records of *R. pravus* may be incorrect. Neumann's and Howard's records, however, will not be included, not only because they are dubious from the systematic point of view, but because in many cases exact localities are not given.

Some of the specimens identified as *R. bursa* by Lewis (1932) have on examination proved to be *R. kochi* and *R. hurti*, not *R. pravus*. Wilson (1954) states that the measurements of the conscutum of *R. hurti* are in some cases almost identical with those of *R. pravus*, but otherwise the species are relatively easy to differentiate. The capitulum of the male, as in *R. bursa*, is compact and the lateral angles of the basis capituli are far forward. The eyes are fairly small and either flat or only slightly convex, the cervical fields are not sharply defined and the punctations are more even than those of *R. pravus*. In the female of *R. hurti* the cervical fields are broader and the punctations rather larger and more even in size than those of *R. pravus*.

From the descriptions and illustrations given it appears that *R. mossambicus* Dias, 1950, and *R. piresi* Dias, 1950, come within the range of variation of *R. pravus* and thus fall as synonyms of this species.

The synonymy of *R. pravus* Dönitz, 1910, is now as follows: *R. neavei* Warburton, 1912; *R. neavei punctatus* Warburton, 1912; *R. punctatus* Warburton, 1912 (Dias, 1951); *R. mossambicus* Dias, 1950; *R. piresi* Dias, 1950.

R. pravus features as *R. neavei* in the publications of Lewis (1934, 1939), Lewis, Piercy & Wiley (1946), Wilson (1946, 1950), Zumpt (1949), Rousselot (1951), Rageau (1953), Theiler & Robinson (1954); as *R. neavei punctatus* in Lewis (1931), Sousa Dias (1948), Santos Dias (1950*c*), Wilson (1946, 1950) and as *R. bursa* in Neumann (1897, 1901, 1911), Newstead *et al.* (1907), Howard (1908), Schwetz (1927), Bequaert (1931), Lewis (1939) ex parte and Santos Dias (1950*c*).

Laboratory rearing

R. pravus, which is a three-host tick, is not always easy to rear under laboratory conditions. Engorged females were kept in an incubator at 25–27° C. until the eggs they had laid hatched, and the larvae were then kept at room temperature, which fluctuated between 16 and 23° C. Similarly, engorged larvae and nymphae were placed in the incubator to moult and then transferred to a cupboard at room temperature for storage until they were fed again. Either rabbits or cattle were used as hosts and the ticks fed on their ears. Accurate records of the periods required by larvae, nymphae and adults for hardening (frequently taken to be approximately a week) were not kept, but the periods required for the other phases of development were as follows: preoviposition period 4–6 days; larvae hatch in 28–33 days; larvae engorge in 4–8 days; larvae moult in 7–12 days; nymphae engorge in 5–11 days; nymphae moult in 16–19 days; adults engorge in 7–19 days.

Survival periods

Accurate data are not available.

Distribution and hosts

R. pravus is widely distributed in Eastern, Central and Southern Africa and has been collected from numerous hosts. It does not appear to extend far into West Africa and is absent in North Africa. Detailed accounts of its distribution in Kenya will be given by Wiley and in South Africa have been given by Theiler & Robinson (1953).

In Kenya, where *R. pravus* was originally identified as *R. neavei*, it appears to be scattered throughout the Northern Province and the Coast Province. Moving westwards, it is very plentiful in the Ukamba area of the Central Province and is present in many of the drier parts of Masai, particularly round Narok. It is absent in the Highlands and in the Nyanza Province.

Theiler & Robinson (1953) state that the records of the occurrence of *R. pravus* in South Africa are not entirely accurate. At one time it was not recognized as a separate species and 'was undoubtedly listed at times as a very coarse *R. appendiculatus* or as a doubtful *R. capensis*, or an *R. sp. "eyed"*. Howard's *R. bursa* (*Ann. Transv. Mus.* 1, 6, p. 130; plate VIII, fig. *h*; plate X, fig. *h*) is in all probability this species.' From the available data, however, its distribution—to quote from their publication

falls roughly into two main blocks. I. The block associated with the *Bushveld* vegetation, reaching northwards into Rhodesia and westwards into the Kalahari of Bechuanaland. Actual records exist from areas in the districts of Zoutpansberg, Pietersburg, Potgietersrust and Waterburg in the Northern Transvaal, in Schweizer Reneke and Wolmaranstaad in the western Transvaal; in Kimberley, Philipstown and Hanover, with an outlier at Prieska in the north-western Cape; and reaching via *Bushveld* incursions into the Brokenveld of Bethulie, Rouxville, Zastron, Edenburg, Fauresmith, Southern Bloemfontein and Thaba 'Nchu, in the Orange Free State. All areas below the 20 in. average annual rainfall. II. The block in the eastern Cape province in the districts of Humansdorp, Uitenhagen, Jansenville, Somerset East and Adelaide.

As yet Kenya and South Africa represent the only two areas in which the distribution of *R. pravus* is known in some detail. Records from other regions are relatively sparse. These are quoted in full in Table 1 (except the records from Neumann (1897, 1901, 1911) and Howard (1908), which have already been discussed).

In addition to the animals recorded above, *R. pravus* adults have been collected from the following hosts: oribi (gwape), hartebeest and *Lepus whytei* (Wilson, 1950, syn. *R. neavei* and *R. neavei punctatus*); *Poëlagus* sp. and *Lepus saxitilis*; the steenbok, nyala, Bright's gazelle; Grant's gazelle, oryx, buffalo, giraffe, sable antelope and waterbuck and the spotted hyaena, leopard, lion and genet; immature specimens have been collected from field mice, *Mastomys coucha*, *Aethomys namaquensis auricomis* and *A. chrysophilis* and the elephant shrews *Elephantulus rupestris* and *Nasilio brachyrhynchus* (Theiler & Robinson, 1953). Wiley also records the gerenuk, or Waller's gazelle, and Hoogstraal (1954*b*) a shrike, '*Telephonus*', as hosts of the adults.

Hitherto *R. pravus* has always been regarded by workers in East and South Africa as a relatively 'dry-country' species of tick. The records of its occurrence

Table 1

Synonym	Host	Locality	Authority
		NIGERIA	
R. neavei punctatus	Cattle	*Western Province* Benin	Gamble, 1952 (private communication to the Director, I.B.E.D.)
—	Cattle	*Cameroons* Jakiri, Bakanke	Unsworth, 1952
		ANGLO-EGYPTIAN SUDAN	
—	Domestic animals and hares principally	Equatoria: most abundant in the eastern parts of the province, decreasing in numbers towards the Nile and absent west of the Nile	Hoogstraal, 1954 *a*
		ETHIOPIA	
—	—	Diredawa	Zumpt, 1942
		FRENCH SOMALILAND	
—	—	Ambouli	Hoogstraal (1953)
		BRITISH SOMALILAND	
R. neavei	Camel, sheep and goat	Las Anod, Danot, Wadere, Galadi, Las Dureh, Burao, Gararho, Legib, Dambareh, Libah Hele, Ainabo Wells, Boramo	A. J. Wiley (personal communication)
		ITALIAN SOMALILAND	
R. neavei	Lesser kudu	Gedful	A. J. Wiley (personal communication)
		BELGIAN CONGO	
		Kivu	
R. bursa	Bovine	Kasongo	Newstead *et al.* 1907
R. bursa	*Tragelaphus scriptus*	Kibombo	Schwetz, 1927
		Costermansville, Kikumba	Theiler & Robinson, 1953, 1954
		Ruanda-Urundi	
R. bursa	Cattle	Nya Lukemba	Newstead *et al.* 1907
R. neavei	Cattle	Kisenyi-Kibere, Ijenda, Rwankuri	Rousselot, 1951
		Nyakatale, Ruhengeri, Bukamba, Kisenyi, Kingogo, Murombi, Mucucca	Theiler & Robinson, 1953, 1954

Table 1 (*continued*)

Table 1 (*continued*)

Synonym	Host	Locality	Authority
		Katanga	
Identified as *R. bursa* by Schwetz, 1927, as *R. neavei* by Bequaert, 1931	*Phacochoerus aethiopicus* —	Bukama	Schwetz, 1927; Bequaert, 1931
—	—	Bukama, Elizabethville, Mura River	Theiler & Robinson, 1953, 1954
		UGANDA	
		Western Province	
—	*Oreotragus* sp.	Ishingiro Hill, Ankole	Nuttall Collection no. 3895 A (Warburton, determined)
		Gulu, Patiko	Theiler & Robinson, 1953
		Eastern Province (Karamoja)	
R. neavei	*Rhynchotragus kirkii*	Lorengidwat	Theiler, 1952 (personal communication)
R. neavei	*Strepsiceros imberbis*	Lorengidwat	Theiler, 1952 (personal communication)
—	*Lepus capensis*	Namalera, Namalera River, Lorengidwat, Lokichar, Komolo area	Theiler, 1952 (personal communication)
		Jie, Lolito, Ngoropo	Theiler & Robinson, 1953
		TANGANYIKA	
		Lake Province	
—	*Gazella thomsonii*	Banagi Hill, Trina River (near Nata), Ngeta and Guta (near Musoma)	Author
		Northern Province	
		Arusha	Zumpt, 1942
—	*Gazella thomsonii*	Yaida Plains	Author
		Central Province	
		Mkalama, Kondoa-Irangi, Kilimatinde, Mpwapwa	Zumpt, 1942
—	Common duiker	Manyoni district	Author

Table 1 (*continued*)

Synonym	Host	Locality	Authority
		Eastern Province	
—	Oryx	Southern borders of Masai steppe	Nuttall Collection (presented by Dönitz, 1911)
		Morogoro	Zumpt, 1942
		Southern Highlands	
—	Cattle	Kalengo, Kimande	Author
		MOZAMBIQUE	
R. mossambicus	Buffalo	—	Dias (Santos), 1950 *a*
R. piresi	*Paraxerus cepapi auriventris*	Lumasse (near Guija, Gaza district)	Dias (Santos), 1950 *b*
R. punctatus	*Lepus capensis capensis*	Matola (near Marracuene, Lourenco Marques district)	Dias (Santos), 1951
—	*Tragelaphus angasii* and *Cephalophus natalensis natalensis*	Maputo (Lourenço Marques district)	Dias (Santos), 1951
R. neavei	*Nesotragus livingstonianus zuluensis*	Chicualacuala	Dias (Santos), 1954
		NYASALAND	
R. neavei punctatus (Types)	Kudu	Near Fort Mlangeni	Warburton, 1912
	Impala	N.W. shore of L. Nyasa	Warburton, 1912
	Reedbuck	Rukuru River Valley	Warburton, 1912
R. neavei	Roan antelope	Near Marisba	Warburton, 1912
R. neavei	Bush buck, wart-hog, *Lepus* sp., cattle, grass	No locality references	Warburton, 1912
		Numerous records without any exact locality references	Wilson, 1950
		Fort Hill, Chinunka, Chitala	Theiler & Robinson, 1953

Table 1 (*continued*)

		NORTHERN RHODESIA		
R. neavei (Types)	Eland	Tasangazi River, Luangwe Valley	Warburton, 1912	
R. neavei	Bush-pig, bush-buck, impala, kudu, man	No locality references	Warburton, 1912	
—	—	Wayetwekas Village (15 miles south of Fort Hill), Isoka, Musagamas, Chinga, Chinsali, Ndola, Nteka, Namwala, Machile, Choma-Macha, Mongu	Theiler & Robinson, 1953, 1954	
R. neavei	—	Isoka, Chinga	Theiler & Robinson, 1954	
		SOUTHERN RHODESIA		
		West Nicholson, Bulawayo, Nyati, Tjankwe	Theiler & Robinson, 1953	
		SOUTH-WEST AFRICA		
		Caprivi Zipfel		
		Kalundu	Theiler & Robinson, 1953	
		BECHUANALAND		
		Eastern Bechuanaland		
		South of Kazungula, Mahalapye, Palapye	Theiler & Robinson, 1953	
		North-western Bechuanaland		
		Dikate, Tatamoga, Kobe (Maun district)	Theiler & Robinson, 1953	
		ANGOLA		
R. neavei punctatus	—	Chiumbo, Lunge, Luimbale, Mungo in Bailundo, Cassengue, Cuito in Quipeio, Cussava, Huambo, Lépi in Caála, Vila da Ponte in Ganguelas, Humpata, Lubango	Dias (Sousa), 1948 (Quoted in Theiler & Robinson, 1953, 1954)	

are most numerous in areas with an average annual rainfall of under 30 in., as in British Somaliland, parts of Kenya and parts of South Africa. Broadly speaking, the vegetation in these areas appears to be mostly of the desert grass savannah or tall grass savannah type (Bushveld or dry Parklands—Theiler & Robinson, 1953). There are, however, also records, though they are not so numerous, from areas in the Congo, Tanganyika and Northern Rhodesia which have an average annual rainfall of well over 30 in. and from Benin, Jakiri and Bakanke in Nigeria where the rainfall averages over 60 in. per annum. Benin, in particular, is in a high forest zone with high atmospheric humidity and is not an area in which one would expect to find *R. pravus*.

Little is known about the influence of the various environmental factors on the tick, though it is obvious that both humidity and vegetation are important. It is possible that, in some cases where *R. pravus* is recorded from areas which one might think would be too wet for it, the distribution of rainfall is poor and the dry-season atmospheric humidity low, resulting in the production of a relatively xerophilous type of vegetation. This is suggested by the comments of Rosevear (1953) on the growth of vegetation in Nigeria. He says that 'this factor of atmospheric dryness together with the absolute length of the dry-season, defined as comprising all months with a mean monthly rainfall of less than 1 in., appear to have a far greater influence upon the vegetation than the total precipitation has, and one may expect to find a more arid type of growth in a locality with a high rainfall and low dry-season humidity than in one with a low rainfall and high humidity'.

Another point worth noting is that in some areas in which one would not expect the tick to occur, e.g. Benin, the collections were made from cattle. It is possible that these merely represent introductions, perhaps on slaughter stock, and that the tick is not actually established in these areas.

Disease transmission

R. pravus (syn. *R. neavei*) was proved by Lewis *et al.* (1946) to be capable of transmitting *Theileria parva*, the causal organism of East Coast fever of cattle, under laboratory conditions. It does not, however, appear to act as a permanent reservoir of this disease in the field since East Coast fever is normally absent from those areas in which *R. pravus* is found. This may be due to the fact that the larvae and nymphae of this species normally feed on small rodents such as mice and elephant shrews and not on stock.

The author wishes to thank the Director, East African Veterinary Research Organization, for permission to publish this paper and the following workers for their generous assistance: Dr G. Wilson, Kaduna, Northern Nigeria, for first drawing her attention to Dönitz's specimens of *R. pravus*; Dr G. Owen Evans and Mr E. Browning of the Zoology Department, British Museum (Natural History) for permission to examine the types of *R. neavei* and *R. neavei punctatus* and for the loan of specimens; Dr G. Theiler, Onderstepoort, for much advice, the loan of specimens and permission to quote unpublished data on the distribution of

R. pravus; Dr H. Hoogstraal, U.S. N.A.M.R.U. 3, Cairo, for helpful advice; A. J. Wiley, Veterinary Research Laboratory, Kabete, for information on the distribution of this tick in British Somaliland and Kenya and permission to quote unpublished data; Dr J. A. T. Santos Dias, Maputo, Mozambique, for the loan of specimens; and workers in the field, particularly A. C. Brooks, who have sent in collections of ticks for identification.

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(*MS. received for publication* 28. v. 1955.—Ed.)

THE MORPHOLOGY OF THE BRITISH PROSTRIATA, WITH PARTICULAR REFERENCE TO *IXODES HEXAGONUS* LEACH. III

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(With 69 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	261
II.	The spiracle—review of literature and methods	262
III.	Spiracle-cuticle relationship	263
IV.	The spiracle in the female	264
V.	Haller's organ—introduction	272
VI.	Material and methods	273
VII.	The structure of Haller's organ in *Ixodes hexagonus* Leach	274
VIII.	Haller's organ in other Prostriata	276
IX.	Discussion	293
X.	Summary	305
	References	306

I. INTRODUCTION

The present account is a continuation of earlier studies (Arthur, 1951, 1953*a*, *b*), which have been undertaken to amplify and correct previous interpretations of some aspects of tick morphology. *Ixodes hexagonus* Leach, 1815, has been selected, as previously, as the basic type and for the more detailed studies. The results embodied in the present paper deal with the structure and possible functioning of the spiracle, and with the detailed structure of Haller's organ with more particular reference to the British species. However, in order to substantiate some of the arguments put forward in connexion with Haller's organ, it has been considered desirable to examine a large number of non-British species. A more detailed survey of the latter is proceeding and will, it is hoped, be reported in due course. In preparing this paper I was fortunate in being able to obtain material from a large number of sources. In addition to those whose assistance has been acknowledged previously (Arthur, 1953*b*), the following correspondents responded generously in supplying me with required specimens: Mr Glen M. Kohls, Mr J. D. Gregson, Dr F. C. Bishopp, Dr R. E. Snodgrass, Dr Gertrud Theiler, Dr F. Zumpt, Dr Harry Hoogstraal, Dr Marc André, Dr Colas-Belcour, Mr Niels Haarløv, Dr Tuxen, Dr Aellen, Professor O. Theodor, Dr Campbell, Dr Cameron and Dr Guy Morison. To all concerned I am indebted for their kindness and their ready assistance. Dr P. Tate very kindly read through the manuscript and offered many suggestions which have improved the paper. The work was completed when the writer was in receipt of a Leverhulme Research Award, and he acknowledges his thanks to the Trustees for their financial assistance.

II. THE SPIRACLE—REVIEW OF LITERATURE AND METHODS

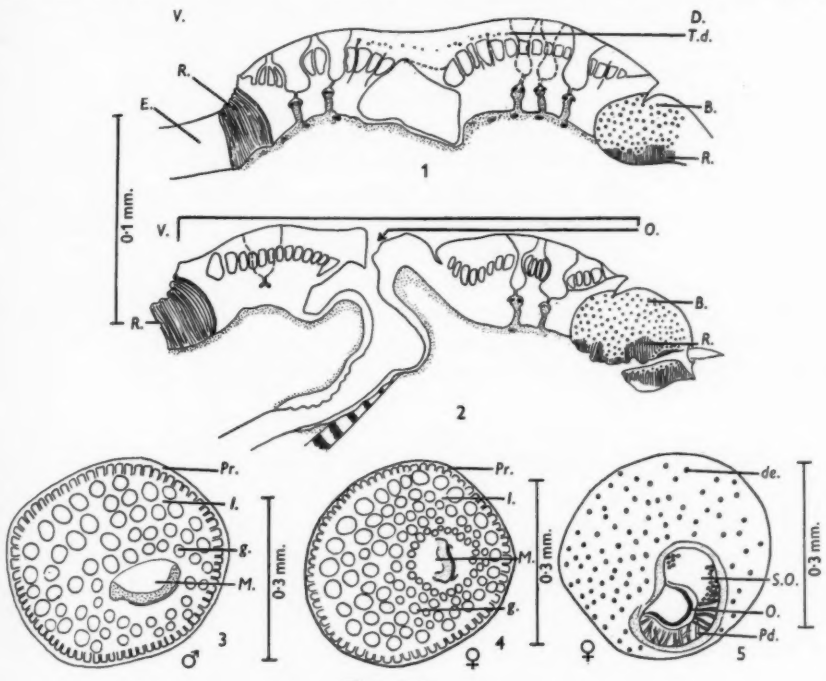
The earliest reference to the structure of the spiracle of ticks (presumably that of *Ixodes ricinus* L.) is contained in a paper by Batelli (1891), which is illustrated by one figure. Bonnet (1907), in his anatomical studies on ticks, referred to the spiracles of *I. hexagonus*, and in this respect it is interesting to note that he also figured ducts leading from the epidermis through the cuticle to the base of the pyriform spaces. His interpretation of this structure in *I. hexagonus* was also accepted by Schulze (1923). Nuttall, Cooper & Robinson (1908*b*) commented on the little attention devoted to the spiracular structure in the Ixodoidea, and no complete description or published figures of the spiracle had until that date been available. These workers dealt with the spiracle of *Haemaphysalis punctata* Canestrini & Fanzago, in which they recognized the sieve-plate arrangement of the outer membrane, the intermediate pedicellar layer, the thicker basal layer penetrated by ducts and a 'large chamber of irregular shape which we have termed the atrium'. Nordenskiöld (1908) and Samson (1909) described the spiracle in *Ixodes ricinus*, and Robinson & Davidson (1913) dealt with that of *Argas persicus* (Oken). These workers elaborated the work of Nuttall *et al.* (1908*b*) with reference to the atrium which they observed was 'a somewhat elongated cavity the lumen of which is contracted about its middle'. It was further noted that the cuticle of the 'bulge' was ridged, and that proximally a muscle from the dorsal body wall was inserted on the upper wall of the lumen. Mellanby (1935) has dealt summarily with the structure of the spiracle of *Ornithodoros moubata* Murray, but adds nothing to our previous general knowledge of structure. However, this paper is of interest from a functional aspect in as far as Mellanby's experimental data permitted him to draw the conclusions that (1) there is a physiological reaction which permits the opening and closing of the spiracles, and (2) the increase in rate of water loss which occurs when the spiracles are permanently open implies that such loss is due to evaporation principally from the tracheal system and that the cuticle is relatively impervious. One further point of interest is that Mellanby believed that from a morphological point of view the ostia in *O. moubata* can be opened and closed. Browning (1954) has reviewed the structure of the spiracle in *O. moubata*, to which specific attention is drawn later. Douglas (1943) has given an account of the spiracle of *Dermacentor*, and in this species Stiles (1910) has utilized the spiracular plate as a diagnostic specific character. Snodgrass (1952), after reviewing the work of Stiles (1910), Douglas (1943), Nuttall *et al.* (1908*b*), Nordenskiöld (1908) and Falke (1931), expressed the opinion that the structure of the spiracular plate clearly needs further study. Existing descriptions of the spiracles as a whole in *Ixodes hexagonus* are meagre, and, since it would appear to be so different (Bonnet, 1907; Schulze, 1923) from comparable structures in ticks of the same and other genera, a re-description of its morphology is given here.

The anatomy of the spiracle in the present studies on *I. hexagonus* was investigated from dissections of freshly killed specimens, from caustic potash preparations and from vertical, transverse and longitudinal sections. For fixation, hot Bouin's

alcoholic fixative was satisfactory. Carnoy's glacial acetic-absolute alcohol-chloroform mixture also yielded good results. Sections were stained either with haematoxylin or Mallory's triple stain.

III. SPIRACLE-CUTICLE RELATIONSHIP

The adults and nymphs of *I. hexagonus*, in common with other ticks, have a single pair of spiracles which in unfed specimens are located on the latero-ventral surface of the opisthosoma immediately behind the coxae of the last pair of legs.



Figs. 1–5

Fig. 1. Longitudinal section of spiracle, outside level of ostium.
Fig. 2. Longitudinal section of spiracle, through the ostium.
Fig. 3. Surface view of male spiracle.
Fig. 4. Surface view of female spiracle.
Fig. 5. Internal view of female spiracle.

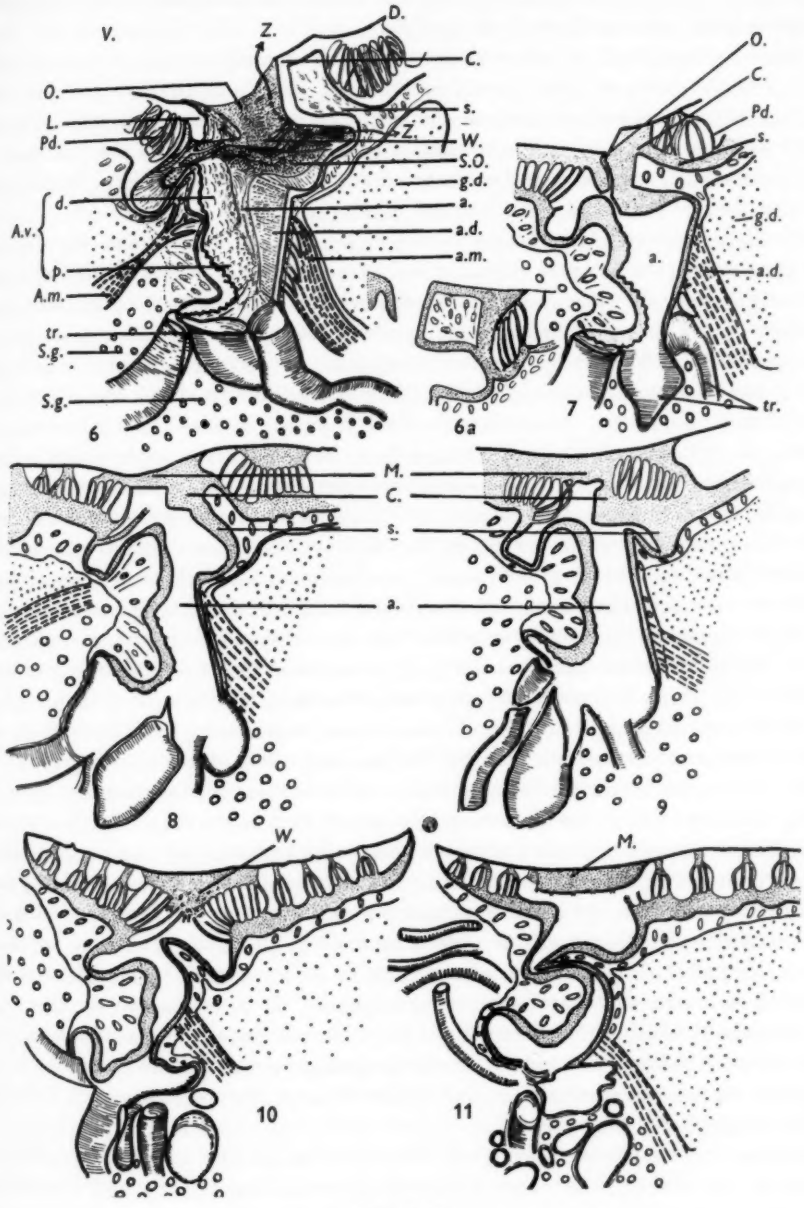
Key to abbreviations pp. 305, 306.

These spiracles are round in the male and female, and oval in the nymph where the longitudinal axis lies transversely to the major axis of the tick's body. Spiracles are absent in the larvae. In the female and nymph the spiracles are surrounded by the extensible alloscutum, which consists of finely ridged thin epicuticle with an inner and outer endocuticle. This arrangement is also applicable to the spiracle, where the endocuticle is sclerotized and visible beneath the superficial spiracular plate. The epicuticle here is not finely ridged. Lees (1952) showed that in the newly

metamorphosed tick the outer alloscutal endocuticle is thick and its inner counterpart is virtually absent: the latter, however, starts to thicken before feeding has commenced and continues to do so during the feeding period. The inner endocuticle of the spiracle does not, however, thicken to the same extent, and there appears to be little or no increase in this thickness from the unfed to the gorged state. In the male the extensible integument around the spiracle is much reduced in association with the greater development of non-extensible plates. Transverse sections, stained with Mallory's triple stain, show that the cuticle adjacent to the ventro-lateral margin of the spiracle stained blue, and this was overlain by a thin, ridged, non-stained epicuticle, reminiscent of the female alloscutum. Adjoining this blue belt ventrally (Fig. 1) is the unstained yellow cuticle of the epimeral plate, which in turn ventrally is followed by a further 'blue belt' and adjoins the median ventral plate. Dorsolaterally the spiracular plate has a concave margin, and into this a bulge of the alloscutal cuticle fits. On the outer surface a notch partially separates the plate from the cuticle. The 'bulge' of the cuticle stained to a brick red colour (stippled in Figs. 1 and 2), except for (i) a narrow band of unstained yellow cuticle immediately beneath the flat, thin non-folded epicuticle and (ii) a somewhat thicker, irregular 'blue' staining layer above the hypodermis. The blue-staining layer is presumably the inner endocuticle, so that the degree to which this is developed, at least in the male, will vary according to its location on the body. The arrangement of these layers around the spiracle is indicated in Figs. 1 and 2.

IV. THE SPIRACLE IN THE FEMALE

The spiracles in both the male (Fig. 3) and female (Fig. 4) are more or less rounded, with a small ovoid area, the macula, situated antero-ventrally. This macula is more heavily sclerotized than the remainder of the plate and somewhat darker in colour. The macula is cleft by a horizontal crescentic-shaped slit, the ostium. Apart from this the spiracle plate is smooth, rigid and pigmented. It is asymmetrically depressed, and the concavity is deepest around the ostium (Figs. 6, 7), i.e. towards the antero-ventral portion of the plate. The border of the spiracle is formed by a ring of regular 'cuboidal cells', whose peripheral thickened boundaries form a distinct peritreme. Between the latter and the macula, when seen in surface view, there are clear round spaces, or goblets, surrounded by denser interspatial cuticle. Towards the lower edge of the spiracle there are two to three rows of goblets, which increase in size towards the periphery; similarly, there are five rows of goblets from the macula to the upper edge. Perforations in the surface of the goblets have been reported by some authors (Nuttall *et al.* 1908*b*; Falke, 1931). More recently, Browning (1954) has indicated that no definite porous structure has been observed in *Ornithodoros moubata*. This he showed by injecting the tracheal system with cobalt sulphide (Wigglesworth, 1950), when sectioned there was no evidence of this chemical penetrating the outer membrane such as would happen if the outer plate were porous. In uncleared material of *Ixodes hexagonus* perforations may appear to be present but in sectioned material perforations could not be detected; on the contrary, in a few goblets the outer surface, probably consisting in the main of epicuticle, is pushed up into a nipple-like projection without



Figs. 6–11

Fig. 6. Reconstruction of half spiracle, looking anteriorly (reconstructed from sections).

Figs. 7–11. Sections to show relation between the columella and the macula.

evidence of a break. The illusion of perforations is probably due to the opening of dermal glands into the base of the goblets.

Cleared preparations of these spiracles show, in common with other ticks, that the spiracle consists of three layers, each having its own distinctive pattern. The relationship between these three layers can only be adequately interpreted from serial sections, and from such information it is possible to consider the anatomy of the spiracle and its associated structures under (*a*) the spiracular plate, (*b*) the sub-ostial space, (*c*) the atrial chamber, and (*d*) the trachea.

(*a*) *The spiracular plate.* In the region of the goblets, i.e. outside the macula, the plate consists of an outer cuticular non-extensible layer, which is characterized by being alternately thick and thin. The thinner portion overlies the goblets as seen in Figs. 8 to 13, and the thicker portions, which occupy the spaces between successive goblets, are formed by the coalescing ends of underlying rods or pedicels which merge around the periphery of the goblets. This produces the effect of a reticulate pattern when the plate is observed from the surface. It is important to realize, as Mellanby (1935) stressed, that these pedicels are not septa but columns, so that there is a continuity of space between the goblets. Each pedicel is a diamond shape in cross-section, and their somewhat swollen proximal ends fuse with the thick inner cuticular layer. In other species these rods have been considered by earlier workers as solid structures. In females and males of *I. hexagonus*, however, they are tubular and are continuous with tubular extensions which pass through the inner cuticular layer where their open ends abut against the epithelial cells. These pedicellar ducts do not appear to penetrate through the superficial layer of the plate, but cease abruptly about midway through it, as indicated in air-dried sections (Figs. 1 and 14). From stained preparations there is no evidence that cytoplasmic extensions from the hypodermal cells enter into these ducts.

The inner cuticular layer is structurally uniform where it underlies the goblets, being concave at their bases, into which narrow ducts open (Fig. 14). Each duct then passes inwards through the cuticle, becoming wider about midway, at which point there is a stellate cuticular thickening. Cytoplasm from the underlying epithelium extends into the wider proximal portion, but does not penetrate into the finer distal end. Nordenskiöld (1908) ascribed a glandular function to these ducts, Nuttall *et al.* (1908*b*) considered them to be sense organs, and Falke (1931) referred to them as 'champagner-propforganen'. It is certainly true that the underlying epithelium is thicker here than elsewhere in the opisthosoma, but the repeated failure to identify anything analogous to nervous elements or to nervous connexions prompts me to consider them as tegumental glands as originally suggested by Lees (1948).

Earlier workers have given little consideration to the relationship existing between the macula, the upper and lower lips and the ostium, and one writer (Nuttall *et al.* 1908*b*) described it thus: 'The portion of the spiracle beneath the macula... is occupied by a columnar mass of connective tissue and muscle fibres (= the columella) running up from the soft structures underlying the spiracle.' I propose to give the term 'columella' to that portion of the macula which constitutes the upper lip and, as will be seen later, its connexion to the inner cuticular

layer will be referred to as the 'stalk'. The peripheral region of the macula differs from the alternating thick and thin cuticle of the rest of the superficial plate in that it consists of a fairly uniform, thick cuticular layer (Figs. 10, 11). Towards the centre of the macula and in approximate line with the long axis of the ostium this cuticular layer becomes thickened into a wedge-like projection (Figs. 6, 10). At this point it is of interest to note that pedicels arise from the walls of the subostial cavity and pass directly into the thickened macula. These pedicels differ from those associated with the goblets in that they pass individually into the macula without terminal fusion into groups, but nevertheless they still retain their tubular nature. These pedicels are very much stouter and longer than those of the goblet region. The apex of the wedge increases in length in the direction of the ostium and projects inwards into a subostial cavity (Figs. 6, 9), and the aperture or ostium is the result of an asymmetrical division of this wedge (Figs. 6, 7) whereby the bulk of the latter goes to form the upper lip. Meanwhile, the lengthened apex of the wedge fuses with the deep-seated cuticular layer, producing in section a stalk-like effect (cf. Figs. 6, 9 and 10). Such a section is depicted in Fig. 6*a*, which occupied an intermediate position at about the level of 'ZZ' in Fig. 6. The lower lip is smaller and consists of an intucking of the free end of the 'split' macula (Fig. 7). The 'stalk' of the 'columella' attains its greatest thickness about halfway along the ostium, and thereafter narrows to its posterior edge with the elimination of the softer tissues within the columella, but the columella and its stalk do not lose their identity for some 30–40μ beyond the actual edge of the opening (Fig. 9). After this the columella and its associated stalk pass gradually into the wedge-shaped form (Fig. 10), which in turn becomes the uniformly thick macula (Fig. 11). Nuttall *et al.* (1908*b*) imply that in *Haemaphysalis* this columella stalk is cylindrical, but in fact, as seen from their figures and from the present series of sections, it is a septum of varying thickness which extends along the length of the ostium and is continuous anteriorly and posteriorly with the cuticle of the macula. For the greater part of its length within the limits of the ostium the columella and its stalk are hollowed and lined with epithelium. Towards the periphery (Figs. 6*a*, 9), the stalk becomes solid, but there is an apparent radiation of the epithelial tissue into the columella (Fig. 6*a*). This epithelium does not extend to any great degree into the macula.

(*b*) *The sub-ostial space.* In *H. punctata*, as described by Nuttall *et al.* (1908*b*), a distinct clear space (pericolumellar space) surrounds the lower portion of the columella. The space lying above the pericolumellar septum (i.e. truly dorsal to it) in *Ixodes hexagonus* is crossed by pedicels (Figs. 7–9) and, as seen in Fig. 7, for example, these pedicels may actually arise from the columellar septum. Hence the space beneath the ostium communicates directly with a dorsal pedicellar space in this species on each side of the septum beyond the disappearance of the 'stalk', i.e. outside the anterior and posterior limits of the aperture. For convenience I refer to the clear space as the subostial space (though in fact its limits extend well beyond the ostium in all directions). This space, when seen in horizontal section, is elongate and lies along the long axis of the tick's body, widening appreciably in the vicinity of the ostium (Fig. 15). For the most part it is shallow, becoming more so towards its

posterior limit, the whole being asymmetrically arranged relative to the ostium (Fig. 16).

(*c*) *The atrial chamber.* The subostial space leads into a narrower inner atrial chamber which is short and tube-like when viewed *in situ*. The earlier description of the spiracle of this species, as given by Bonnet (1907), which was also accepted by Schulze (1923), did not recognize this atrial chamber in *I. hexagonus*, but indicated that the trachea arose from the inner margins of the subostial space. According to Nuttall *et al.* (1908*b*) this atrium is continued downwards as a large chamber of 'irregular shape', and Samson (1909) referred to it as 'sichel formiges Rohr'.

The dorsal and dorso-lateral walls of the atrium are uniformly smooth and flat, whilst the ventral and ventro-lateral walls are thrown into a conspicuous broad lobe, whose long axis lies transversely to the length of the atrium. In the mid-line the lobe is indented, thus dividing it into proximal (*p.*) and distal (*d.*) portions (Fig. 6). Both towards the front and rear end of the atrium this indentation fades out so that the fold appears as a single lobe, becoming smaller forwards and backwards where it merges into the antero-dorsal and postero-dorsal walls respectively (Fig. 13). In these regions the outer dorsal wall and the corresponding lateral areas curve and come to lie between the atrium and the subostial space (Figs. 11, 12); ultimately, this wall at its most posterior limits joins with the base of the ventral lobe so that on the extreme anterior and posterior extensions the atrium is separated from the subostial space (Fig. 12). The walls of the ventral lobe are very much thicker than are those of the dorsal surface, are of a brownish hue rather than of the yellow colour of the latter. The cuticle, particularly of the proximal section of the lobe, is thrown into distinct folds which, however, are not as fine and deep as are those of the epicuticle of the body wall. This, in general, is in agreement with the findings of Samson (1909) in *I. ricinus* and with that depicted for *Haemaphysalis punctata* by Nuttall *et al.* (1908*b*) (plate XXIII, figs. 3, 4), but to which no reference was made in the text. The wall of the atrial chamber is thinner than that of the deepest layer of the spiracular plate, and is devoid of taenidia such as characterize the trachea.

Nuttall *et al.* (1908*b*) wrote that the soft tissue of the columella consisted of a columnar mass of connective tissue and muscle fibres, which have their origin in the soft structures underlying the spiracle. A band of muscle fibres attached to the dorsal wall of the atrium was also observed, and it was indicated that a small portion of these fibres ran up to the superficial layer and were inserted beneath the centre of the macula. Bonnet (1907) figured a strong dorsal muscle which passed

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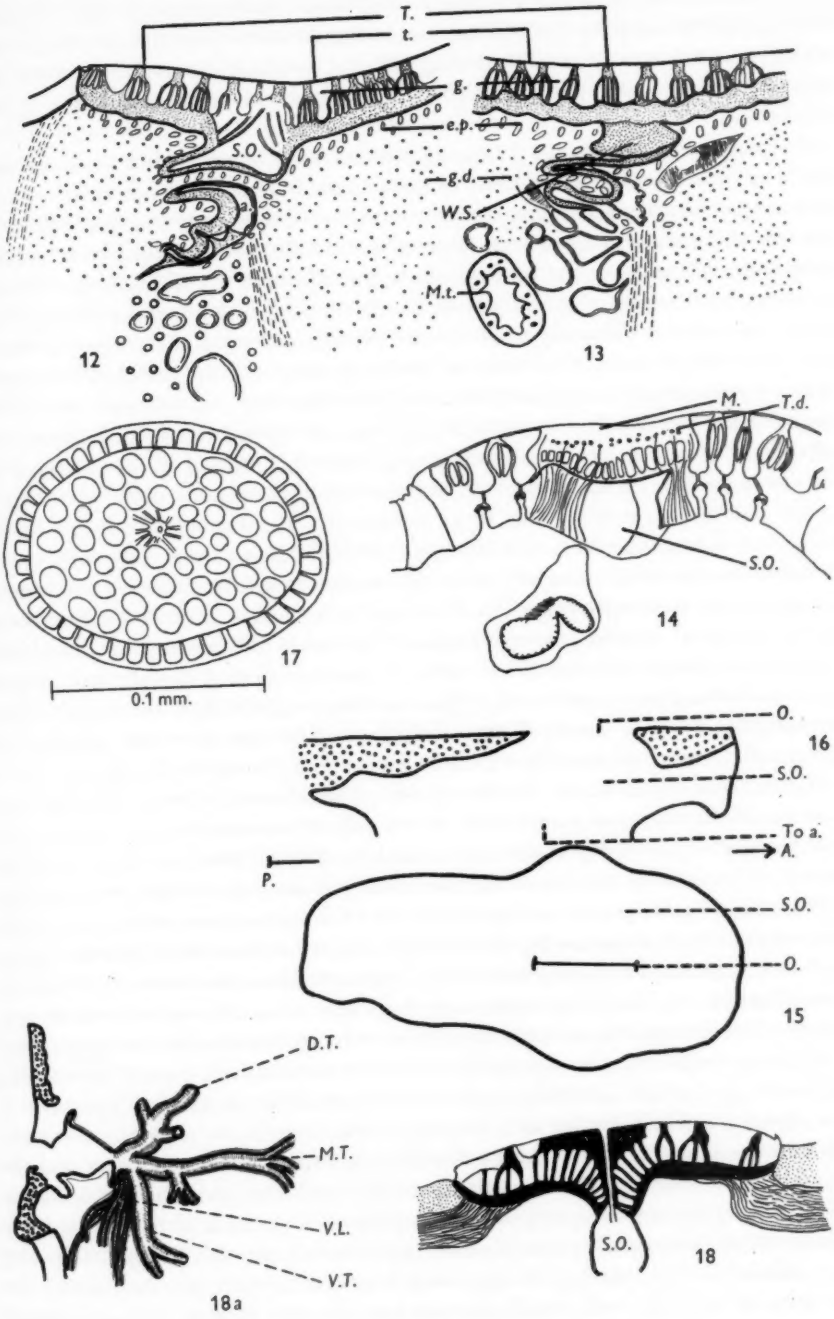
Figs. 12–13. Sections through the spiracular plate at its posterior level. For explanation see text.
Fig. 14. Section through macula of spiracle outside the ostial level.
Fig. 15. Horizontal view of subostial space (reconstructed from sections).
Fig. 16. Vertical longitudinal section through spiracular plate to show position of ostium relative to subostial space.
Fig. 17. Surface view of nymphal spiracle, with macula passing directly to subostial space.
Fig. 18. Section through nymphal spiracle at the level of the ostium. Note the absence of a columella in this stage.
Fig. 18*a*. Diagram showing the mode of origin and disposition of the main tracheal trunks.


T.
t.
g.
e.p.
S.O.
g.d.
W.S.
M.t.
12
13
M.
T.d.
S.O.
14
17
0.1 mm.
O.
16
S.O.
To a.
A.
P.
S.O.
O.
15
D.T.
M.T.
V.L.
V.T.
18a
S.O.
18

Figs. 12–18*a*

into the columella in *Ixodes hexagonus*. Mellanby (1935) is less precise about the insertion of the muscles in *Ornithodoros*, and referred to their attachment to the cuticle near the macula, and Browning (1954) stated that the macula is depressed against the spiracular plate which functions as a pad, without considering the mechanism.

In the present studies the following muscles were observed from sections and whole mounts of the area adjacent to the spiracle in females of *Ixodes hexagonus*: (i) A single muscle band originates from the ventro-lateral body wall just below the spiracle and passes to the ventral lobes of the atrial chamber at or about its mid-ventral line (Figs. 6–8), but on entering into the folds it divides into fine fibrillar bundles. (ii) A series of broad muscle bands arise from the dorsal surface of the body and pass out obliquely to be inserted along the length of the smooth dorsal and dorso-lateral walls of the atrium. Before passing into the cuticular substance of the latter they become fasciated. Mid-dorsally they are attached along the length of the atrial wall, but peripherally they are limited to the inner extremity (cf. Figs. 6–8 and 9–11). The studies of Samson (1909) and Falke (1931) show the existence of a similar band in *I. ricinus*, but in detail they differ with regard to the points of insertion. (iii) In *I. hexagonus* a third muscle band is evident which arises from the base of a latero-ventral infolding of the body integument in unfed females and is inserted on the alloscutum immediately below the ventral margin of the spiracular plate. In *I. ricinus* no reference is made by Samson to such a marginal muscle, whereas Falke (1931) notes the presence of dorsal and ventral components. The earlier work on *I. hexagonus* overlooks all three muscle bands noted in the present work. I have not been able to find any muscle fibres entering into the soft tissues of the columella, and for the most part this consists of epithelial tissue, continuous with that underlying the spiracular plate.

It has been suggested in *Haemaphysalis* (Nuttall *et al.* 1908*b*) and in *Ornithodoros* (Mellanby) that contraction of the columellar muscles and dorsal atrial muscles dilates the cavity of the atrium and possibly at the same time closes the ostium, 'the external margin of the columella overhanging the slit-like opening'. This explanation hinges on the supposition that the columella is mobile on its stalk. From the earlier description this seems highly improbable as the columella septum is of some thickness and relatively hard, and is comparable to the cuticle of the non-extensible scutum, capitulum and legs. The columella septum, moreover, extends in its wedge-shaped form into the macula without the intervention of any articulating device (in other words, the columella and stalk are part of the macula). Any movement of the columella stalk would therefore involve a rotatory action of this wedge for which, as far as I can ascertain, there is no physical allowance. Whilst the spiracular plate may be flexible (Browning, 1954), the facts that the ostium is surrounded by the thick band of the macula and that the columella is a part of the same structure suggest, on morphological grounds at least, that there is no evidence for supposing that the ostium of *Ixodes hexagonus* is regulated in any way. Moreover, on this line of argument it would appear improbable that the elasticity of the body wall would exercise any effective tension, no more than is done on the scutum for example.

No direct occluding muscles have been observed in the spiracles of *I. hexagonus* and earlier workers on other tick species have suggested that the constriction of the lumen of the atrium is effected by the ventral lobe being squeezed against the dorsal wall. This appears to be highly probable, and under such an influence the folds of the ventral lobe become flattened and thus come to lie close against the opposite wall. It has been stated that this is possibly due to the contraction of the dorso-ventral body muscles. This raises the problem of the efficiency of these muscles in this direction. In the unfed female these muscles are not in tonus for they are folded, and would therefore be inadequate for contractile purposes. On the other hand, with the rapid ingestion of blood the gut contents and the body wall become distended rapidly to a remarkable degree. Removal of the water from the blood mass soon turns the ingested blood into a semi-solid sticky mass, which ultimately becomes so highly concentrated that churning movements of the gut are impossible (Lees, 1952), and under these conditions it seems likely that the contraction of the dorso-ventral body muscles will be inhibited to a very large extent. The pressure of the much distended gut will obviously exert a pressure on the atrium and the dorsal and ventral atrial muscles will contract and maintain the lumen of the chamber.

Nuttall *et al.* (1908*b*) stated that there is no ostium in the male or the nymph of *Haemaphysalis punctata*. In males of *Ixodes hexagonus* the ostium is open and in all essentials is similar to that of the female. The musculature is similar in the spiracles of both sexes. The elongate-oval spiracle of the nymph (Fig. 17) has the macula located slightly in front of the middle. Anterior to the central area are two rows of goblets, posteriorly three rows, dorsally three rows and ventrally four rows. Some thirty to thirty-five cuboidal 'cells' form the bounding layer within the rather thickly sclerotized peritreme. No columella is found and the ostial aperture leads directly into the atrium. Muscles attached to the walls of the inner atrial chamber are similar in their distribution to those found in the adult (Fig. 18).

(*d*) *The trachea.* Three major tracheal trunks (Fig. 18*a*) arise from the inner end of the atrium, and ventrally between the lobe and the origin of these three trunks five or six smaller trunks arise. These latter supply the ventro-lateral portion of the body, including the musculature. The ventral trunk passes downwards and mesially, and after a short distance subdivides into external and internal branches; the latter going to the midline and supplying the reproductive system. There is considerable anastomosis of the tracheae from the two sides in and around the midline. Branches pass forward from this internal vessel which ultimately supplies the 'brain'. It is of interest to note that quite large tracheae pass in between the nerve cells of the brain. The external branch supplies branches to the second, third and fourth legs and coxal muscles. The median trunk runs towards the mesal line for a short distance before branching profusely, these pass inwards, forwards and backwards as prominent longitudinal trunks, which in the unfed tick pass through the substance of the salivary gland and in the connective tissue between the caeca of the alimentary canal. The anterior branches diverge and send tracheae to the capitulum and its appendages, the first pair of legs, the oesophagus and pharynx. The dorsal branch turns upwards and backwards on leaving the atrium and subdivides into

longitudinal trunks which diverge posteriorly in such a way that some of them supply the lateral region of the postero-dorsal part of the body and others are directed more mesially.

V. HALLER'S ORGAN—INTRODUCTION

The comparative morphology of the cuticular parts and the phylogenetic significance of Haller's organ in different genera of tick was first investigated by Schulze (1941). According to Schulze, there are two kinds of sensory hairs in the anterior trough ('die Wanne'), and Lees (1948) wrote that in this depression on the fore-leg of the female of *I. ricinus* about seven sensillae are usually present and that their shape is distinctive—'they are 8–20μ long, are very sharply tapered, slightly curved and end in exceedingly fine points'.

Otoliths, reminiscent of the 'sort of auditory organs of Crustacea', within the capsule have been described by Haller (1881) which he called 'Sekretballen'. Similar structures were observed by Schulze (1941), who alleged that they were composed of a tallowy amorphous substance, insoluble in alcohol and xylol. The occurrence of small, crystalline, birefringent granules on the sensillae was also reported by this writer. Bonnet (1907), Nuttall *et al.* (1908*a*) and Samson (1909) failed to detect any of these otolith-like structures; Lees (1948) did not recognize the 'Sekretballen' with certainty, but noted that whilst some of the capsules were empty, others contained dirt particles which had presumably entered through the aperture of the roof of the capsule.

The following table (Table 1) shows those species of the Prostriata in which at least one of the stages of Haller's organ has been described.

Table 1

Species	Haller's organ investigated in			
	Male	Female	Nymph	Larva
I. praematurus P. Sch.	—	—	X	—
I. caledonicus Nutt. & Warb.	—	X	X	—
I. apronophorus P. Sch.	X	X	X	X
I. ricinus scapularis Say	X	X	—	—
I. acutitarsus Karsch	X	—	—	—
Sternalixodes rossianus P. Sch.	—	X	—	—
Sternalixodes cordifer	—	X	—	X
Exopalpiger prisicollaris P. Sch.	—	—	X	—
Endopalpiger steini P. Sch.	—	—	X	—
Endopalpiger luxuriosus P. Sch.	—	X	—	—
I. rasus Neum.	—	X	—	—
I. muntiaci P. Sch.	—	X	—	—
I. ricinus L.	X	X	—	—
I. nivalis Rond.	X	—	—	—
I. autumnalis Leach	—	X	X	X
I. melicola P. Sch. & Schl.	—	X	—	—
I. barbossae P. Sch.	X	—	—	—
I. arboricola P. Sch. & Schl.	X	X	—	—
I. lividus obotriticus P. Sch. & Schl.	X	X	—	—
I. hexagonus Leach	X	X	—	—
I. japonensis Neum.	X	—	—	—

From the information it is evident that Haller's organ in adults, particularly in the females, has received the greatest attention, the immature stages having been largely neglected. Despite this, Schulze (1941) concluded from his available material that the organs in the larvae and nymphs showed agreement in all essential respects with those of adults, and that in the adults the growth of the hairs did not keep pace with the growth of the capsules.

As so little is known of the detailed structure of Haller's organ in all its stages, the objects of the present work were to ascertain: (*a*) whether differences exist in the structure of this organ in the mature and immature stages, a problem which has hitherto been neglected; (*b*) whether 'otoliths' and birefringent particles are secretory products or foreign particles which have entered the capsule; (*c*) whether the structure might provide useful taxonomic characters; (*d*) whether any correlation could be established between the species and the hosts, which might explain such features as host specificity and ecological preferences, and (*e*) whether any observed differences between different stages of the same species might be responsible for varying behaviour patterns.

VI. MATERIAL AND METHODS

Representative samples of all the British Prostriata have been examined, and as far as possible these have been collected in the field. Frequently, however, I have had recourse to preserved material. The organs were examined from both dorsal and lateral surfaces, and for this the tarsi were mounted in either Canada Balsam or polyvinyl alcohol. The sensilla did not show up distinctly in some species, and in these the tarsi were gently boiled in 10% potash for a few minutes. Subsequently, they were either stained in carbol-fuchsin, dehydrated in alcohol and cleared in carbolxylol or alternatively treated for a few minutes with hot pure lactic acid in which was dissolved a small quantity of Solway Green. By the latter method the sclerotized structures stained a bright green, and the sensillae were more clearly defined. Most of the figures were drawn under oil-immersion lens, and measurements made with a calibrated micrometer eyepiece.

Sections of the organ from *I. hexagonus* (females) were also cut. These were fixed in either warm Bouin's alcoholic fixative or in Carnoy's fluid and subsequently infiltrated with either ester wax or celloidin. For staining, Mallory's haematoxylin was found to be useful.

The form, number and arrangement of the sensillae in the anterior trough and in the posterior capsule of Haller's organ are the features which have been considered in the present analysis. These vary, to some extent, from individual to individual, the range of variation being greater in some species than in others. In the case of two British species, the ranges of variation of Haller's organ overlap, thus making diagnosis on this character alone difficult for these species. The shape of the tarsus of the fore-leg distal to Haller's organ, however, provides an additional distinguishing character (Cooley & Kohls, 1945).

The terminology adopted in the past in describing the various parts of Haller's organ has been varied, and to prevent ambiguity the following terms are adopted in the present survey. The posterior capsule in some species possesses only side walls

and is devoid of a roof, and this I have referred to as a 'concavity', the term 'capsule' being retained for species in which the walls and roof are completely formed except for the antero-dorsal aperture. The various processes present in the anterior trough (='accessory pit' of Nuttall *et al.* 1908*a*; 'die Wanne' of Schulze, 1941; 'anterior pit' of Lees, 1948; 'pit-like depression' of Dinnick & Zumpt, 1951, seen in MS.), and in the capsule (or concavity), are referred to as sensory hairs. Those setae or hairs immediately in front of and immediately behind the organ are called bristles, so that we have an anterior bristle group and a posterior bristle group.

VII. THE STRUCTURE OF HALLER'S ORGAN IN *IXODES HEXAGONUS* LEACH

Haller's organ in the female of *I. hexagonus* lies near the distal end and on the dorsal surface of the tarsus of the fore-leg. It consists of an anterior trough and a posterior capsule which is closed except for a small antero-dorsal aperture (Fig. 19). A pair of long and a pair of short bristles are borne on a semicircular plate in front of the anterior trough, and lateral to this group is a single bristle. This is intermediate in size between those on the plate. Two very long bristles and four smaller bristles, between their bases, constitute the posterior bristle group.

The anterior trough is bounded by a horizontal wall on the outer side, and the inner wall ascends obliquely backwards. Six sensory hairs are found in the trough and each lies in a small cuticular socket which surmounts a canal penetrating the cuticle; fine processes, staining with haematoxylin, pass from sense cells immediately beneath the cuticle through the canals and into the bases of the sensory hairs. Morphologically these hairs are of the following types (Fig. 20).

Type I is a single sensilla usually found on an elevation of the cuticle, and lies nearest the outer horizontal wall. It is broad basally, roughly parallel-sided for about half its length and then tapers abruptly to a long, delicate point. The underlying duct is distinctly broader than in Type II.

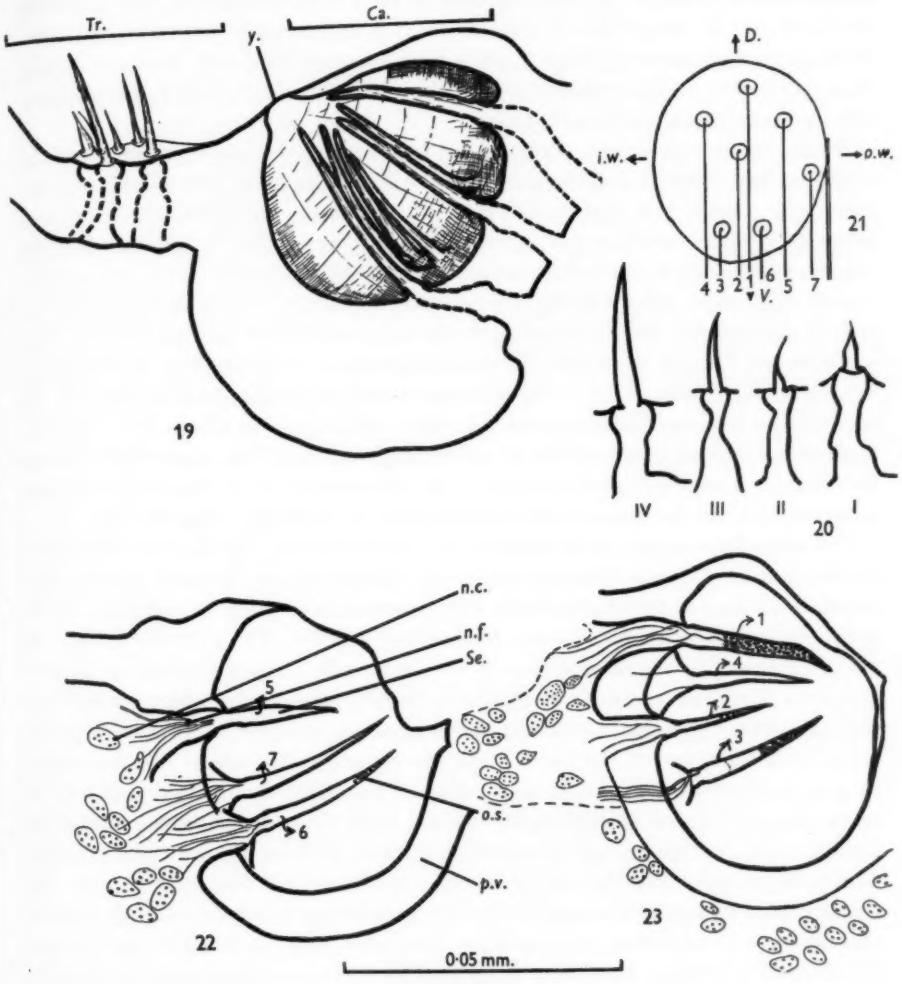
Type II consists of two slightly curving sensillae whose sides taper uniformly from their bases and end in very long, finely pointed tips. They are not borne on a cuticular elevation nor are they as broad basally as that of Type I and frequently one of them is about twice the length of the other. They range from 6 to 12μ in length in *I. hexagonus*.

Type III is represented by two pointed and slightly curved sensillae, whose tips are not as long or as delicate as those of type II. They are broader and taper more gently than in the latter group, and both measure about 15μ.

Type IV is represented by a single sensilla, which exceeds 20μ in length and is located nearer to the mesal margin. This sensilla is very much broader than the others, tapers only slightly and appears to terminate in a short, sharp spine.

The capsule is oval in the nymph, male (Fig. 24) and female (Fig. 19) and in all the long axis is slightly inclined from the vertical. The anterior and posterior walls of the capsule are composed of thickened cuticle, but the curved-up sides and the roof are thin and transparent. Seven thick-walled sensillae arise from slight elevations on the postero-ventral wall of the capsule (Fig. 19). These sensory hairs are fairly uniform in their distribution. The diagram (Fig. 21) represents the most usual

arrangement which I have found in *I. hexagonus*; and the same numbering has been employed in the sections depicted in Figs. 22 and 23. An asymmetrical band of sensory hairs (7, 6, 3 in Fig. 21) is found on the lower part of the capsular wall;



Figs. 19–23

Fig. 19. Haller's organ in *I. hexagonus* female.

Fig. 20. Types of sensory hairs (I–IV) found in the anterior trough.

Fig. 21. Diagram to show the arrangement of the sensory hairs in the posterior capsule: *i.w.*, inner side of leg; *o.w.*, outer side of leg; *D.*, dorsal; *V.*, ventral.

Fig. 22. Section through Haller's organ to show (*a*) the sensory hairs, nerve fibrils, nerve cells at the posterior end of the capsule, and the opaque nature of the distal portion of the sensory hair (dotted).

Fig. 23. Succeeding section to Fig. 22 in the same series. Both sections contain all the sensory hairs within the capsule.

all these hairs are long, about equal in diameter and taper to their apices. A shorter, thinner sensory hair (2) lies median to sensilla 7, and appears to be more variable in position than any of the others when whole mounts are viewed. It may sometimes be seen above or below the level represented by 7 in whole mounts, but in sections the level of 2 is always above that of 7. At a higher level, 5 occupies an intermediate position between 2 and 7, while 4, on a similar level to 5, lies more mesally than any other. In the median line and above the level of the 4th and 5th sensory hairs a single dorsal sensilla (1) arises.

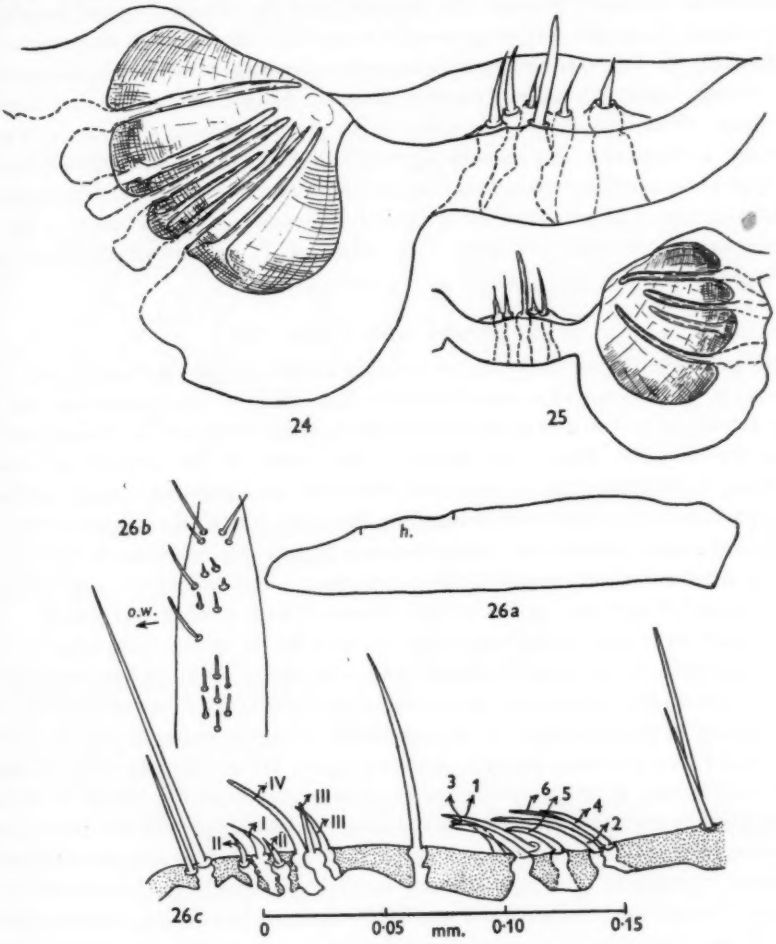
From the figures, drawn with the aid of a camera lucida, it is seen that the sensillae vary in length and thickness *inter se*. The dorsal sensilla is stouter than the remainder, tapers but slightly for most of its length, but at its tip it becomes suddenly narrower and bends downwards beneath the antero-dorsal aperture. The longest sensillae (3, 6, 7) occur in the asymmetrical basal band. Sectioned material reveals that these hairs are slightly constricted basally, before merging into the wall of the capsule. Entire mounts give the impression that the tips of most of the sensillae are bluntly rounded. In certain instances, however, e.g. in the dorsal sensilla of *I. reduvius* and *I. trianguliceps* which lie in the median plane of the capsule, the tips are distinctly pointed. Hairs which arise in planes other than the median are curved, and possibly in entire preparations of the organ their tips are not visible. Longitudinal sections through the capsule of *I. hexagonus* indicate, however, that all the sensory hairs are pointed at their tips (Figs. 22, 23).

The wall of the capsule is thickest on its ventral surface, but the posterior wall is saddle-shaped, i.e. it is thinned out in the median plane. Passing through this thinner cuticle are the ducts which carry nerve fibrils from the sensillae to the group of nerve cells immediately behind the capsule. These fibrils diverge on emerging from the cuticle and pass to the nerve cells. Basally within the sensilla the nerve fibrils fuse to form a single fibre. Distally the sensilla, when seen in longitudinal section, presents a granular and opaque appearance, and I have failed to trace the termination of the nerve fibre. The sensory cells vary in size, are usually oval in shape and when stained have a granular consistency. Haller's organ in the larva (Fig. 25) shows interesting deviations from those found in the adults and nymph. The anterior trough is relatively shorter and carries only five sensillae, whilst the posterior capsule is more rounded and bears only four sensory hairs. The sensory hairs present in the anterior trough are one type I, two type II, one type III and one type IV element—that is, when compared with the latter stages one type III element is lacking. Similarly, in the capsule we have a dorsal sensilla, a ventral sensilla and two lateral elements. A discussion of these differences relative to the behaviour pattern of the larvae and succeeding stages is left to a later date.

VIII. HALLER'S ORGAN IN OTHER PROSTRIATA

Schulze (1941) divided the Prostriata into two groups on the form of Haller's organ in particular. These two groups were the Thecorina or 'capsuled-nosed' ticks such as are exemplified by *I. hexagonus*, and the Plynorhina or 'trough-nosed ticks', where the posterior capsule was not closed dorsally and analogous to a deep trough. Such a broad separation is valid for the British ticks, but is not universal. Of particular

interest in this connexion is the organ in *I. kerguelenensis* André & Colas-Belcour (male) collected from the Kerguelen Islands and from Heard Island. Here the sensory hairs of the organ arise directly from the surface of the cuticle and no anterior trough



Figs. 24–26*c*

Fig. 24. Haller's organ in *I. hexagonus* male.

Fig. 25. Haller's organ in the larva of *I. hexagonus*.

Fig. 26*a*. Tarsus of *I. kerguelenensis* (male). *h*, Haller's organ.

Fig. 26*b*. Haller's organ of *I. kerguelenensis*—dorsal view:—*o.w.*, outer face of leg.

Fig. 26*c*. Haller's organ of *I. kerguelenensis*—drawn from the side.

or posterior capsule is present (Fig. 26*a-c*), nor are they supported mesally and laterally by cuticular elevations forming side walls. The region corresponding to the posterior capsule of other *Ixodes* species bears seven hairs which are curved and correspond to the 'sichelhaar' type of Schulze (1941). The three longest, pointed sensory

hairs are located most posteriorly in a semicircle and sweep forward in a broad curve. In front of these is a single, shorter sickle-hair, and occupying a frontal position are three broader sensory hairs which are likewise strongly curved. No line of demarcation is evident between the 'capsule' and the 'trough' except possibly for the presence of a single very long straight hair which lies nearly in the median line; positionally this has no counterpart in any other *Ixodes* tick which I have examined. The 'trough' bears six hairs. Towards the outer margin and at the rear there are two short, stout hairs (type III) which taper gradually from the base to a point. Adjacent to these is a long sensilla (type IV) slightly curved and pointed distally. The type II are both curved and their apices are drawn out into long, delicate points, whilst the type I is considerably broader basally and is drawn out to a fine tip. A comparison of these sensillae with those of *I. hexagonus* reveals a basic similarity.

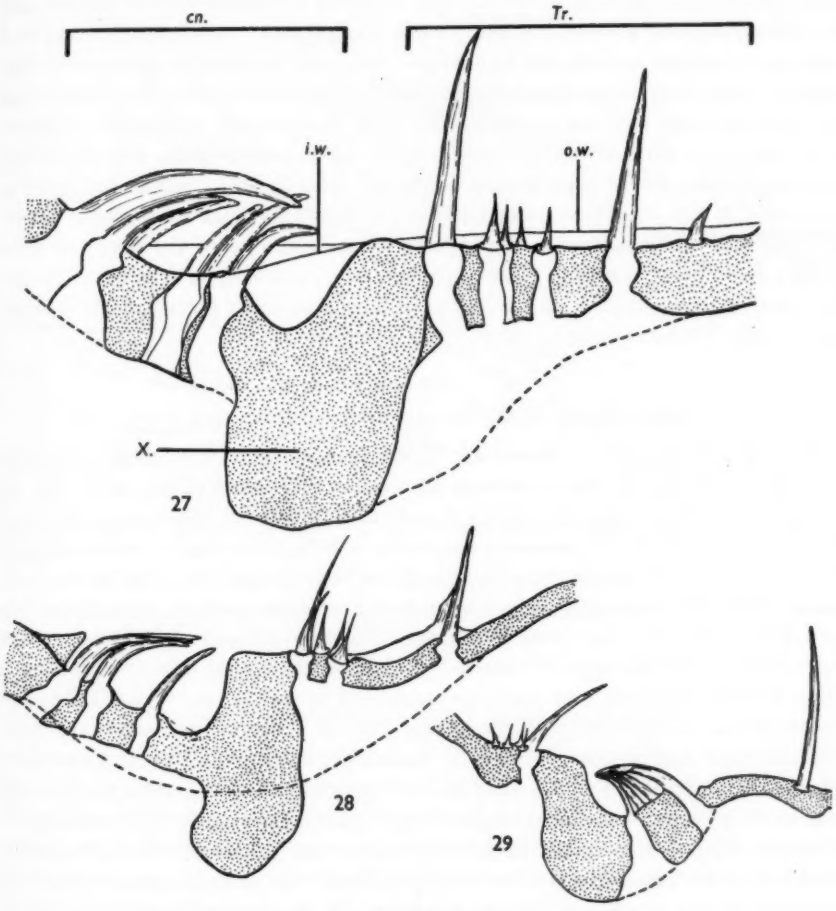
Ceratixodes uriae White, 1852

Schulze (1938) found difficulty in locating Haller's organ on tarsus I in *C. uriae* because of the absence of a closed capsule; later, however, he recognized this and drew attention to the downwardly projecting pillar between the trough and the concavity (Schulze, 1941). The latter in the organs of the nymph and female, according to Schulze (1941), contained secretory granules and a large golden to dark red concretion which was grasped by the sickle hairs. He suggested that this concretion might possibly be a gravitational organ. No reference is made of the form or numbers of the sensory hairs in the trough and concavity, although mention is made of the hairs in the bristle groups of both nymph and female.

The anterior trough in the male (Fig. 27) is bounded on its outer edge by a low horizontal wall. Seven sensillae are present; one, short, stout and pointed apically (type I), lies at the front end of the trough and towards the inner margin of the leg and usually in juxtaposition to a very much longer tapering sensilla. Between these and a long posterior sensory hair are a group of four sharply pointed sensory hairs, which vary in length, but none exceeds a quarter of the length of the long hairs. Usually the more posterior hair is longer than that placed anteriorly, but in some specimens both were equal in length. Both long sensillae are characterized by a gradual tapering to their tips and may be slightly curved. In the material at my disposal I found that the posterior sensilla is constant in position, but the anterior element may either lie opposite the type I sensilla or be closely associated with the group of four short bristles.

A slight elevation in the cuticular framework separates the trough from the concavity, and it is below this that the chitinous framework forms a downward projection which is thicker along the mid-line. The floor of the concavity lies obliquely to the long axis of the leg, and from it seven sensory hairs arise. Of these the most posterior is the stoutest, the most strongly curved and the longest. The bending of the more anterior hairs is most pronounced distally. The anterior portion of the concavity immediately in front of the sensory hairs forms a depression which is deepest along the median line and rises marginally. The walls of the concavity are only partially formed. Both are parallel-sided and extend back almost to the base

of the last posterior sensilla. The posterior bristles are paired and are arranged as follows from the distal to proximal end: two long, two short, two short and two long bristles. I have found no trace of concretions similar to those described by Schulze in the male tick.



Figs. 27–29

Fig. 27. Haller's organ in the male of *C. uriae*.

Fig. 28. Haller's organ in the nymph of *C. uriae*.

Fig. 29. Haller's organ in the larva of *C. uriae*. Note the differences of the sensory hairs in the capsule and trough in Figs. 27 and 29.

Haller's organ in the female is structurally similar to that of the male, but in the anterior trough of one specimen from South Africa two sensillae of equal length were located anteriorly while only three short, sharply pointed ones were arranged in a group. The male may, however, be distinguished from the female in that the former

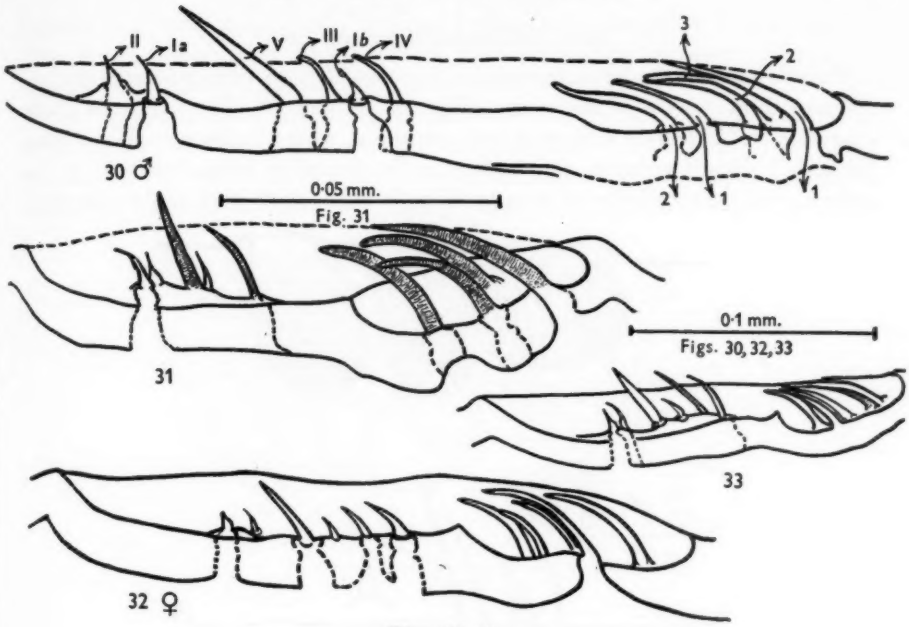
possesses a pointed spur near the tip of the tarsus; in the female it is evident as a rounded protuberance.

Baker & Wharton (1952) wrote that the nymph and larva of *C. uriae* are possibly without this organ. This is incorrect. The nymphal organ (Fig. 28) is similar in general features to that of the adult; the posterior concavity is more deeply sunk into the cuticle and forms a distinct pocket. The posterior bristle group consists of three short bristles set between two longer ones, and the anterior group is of three bristles. Quite frequently the posterior sensilla of the anterior trough is longer than the corresponding anterior member. The more pronounced pocket-like character of the concavity persists throughout the larval instar and the pillar-like thickening, so characteristic of the later stages, is merged into the floor of the concavity as illustrated in Fig. 29. The sensory hairs in the anterior trough are reduced to five: the long anterior component of the later stages and one of the group of short sensory hairs being wanting. Four sensillae, all of which appear to have rounded tips, are found within the concavity; as in later stages the posterior one is longer and broader than the others.

Ixodes (*Eschatocephalus*) *vespertilionis* Neumann 1902

All stages of this species were available for examination. In all stages the organ is elongate and lies within a common depression. The organ in the male (Fig. 30) is very much longer than that of the female (Fig. 32) and, in the specimens which have been examined, the posterior concavity is little more than a continuation of the anterior trough. In the female (Fig. 32) and the nymph (Fig. 33) this feature is also evident; the distinction between the trough and concavity is, however, a little more emphasized by a low transverse ridge. The same components are present in all three stages, but the type IV sensilla is appreciably longer in the male than in either female or nymph, and the same appears to be true for the type III elements. Five sensillae occur in the posterior capsule. These are all of the 'sichelhaar' type, and are longer and broader in the male than in the female. In Fig. 30 the numbers refer to the appearance of the sensillae as they occur from the outer to the mesal surface of the leg. In the larval organ (Fig. 31) both the trough and concavity are relatively deeper than in the succeeding stages, and the distinction between the trough and the concavity is more clearly defined. As in *I. hexagonus*, there is a reduction in the number of the sensory hairs. In the trough one of the type III sensillae is lacking, while in the concavity there are four sensillae which are symmetrically arranged about the long axis. Examination under an oil-immersion lens shows that these sensory hairs are very finely ridged around their circumference. There appears to be no wall along the outer side, but a long low wall extends along the mesal surface from the anterior to the posterior end of the organ.

In all the known *Ixodes* ticks infesting bats (*I. simplex* Neumann (females and nymphs) and species close to *I. simplex*, *I. simplex africanus* (female) and in *I. spiculae* Arthur (male) (Arthur, in preparation for publication)) the form of Haller's organ conforms to that described for *I. vespertilionis*.



Figs. 30–33

Fig. 30. Haller's organ in the male of *I.* (*Eschatocephalus*) *vespertilionis* (for explanation of figures see text).

Fig. 31. Haller's organ in the larva of *I.* (*Eschatocephalus*) *vespertilionis*.

Fig. 32. Haller's organ in the female of *I.* (*Eschatocephalus*) *vespertilionis*. Drawn to the same scale as Fig. 30.

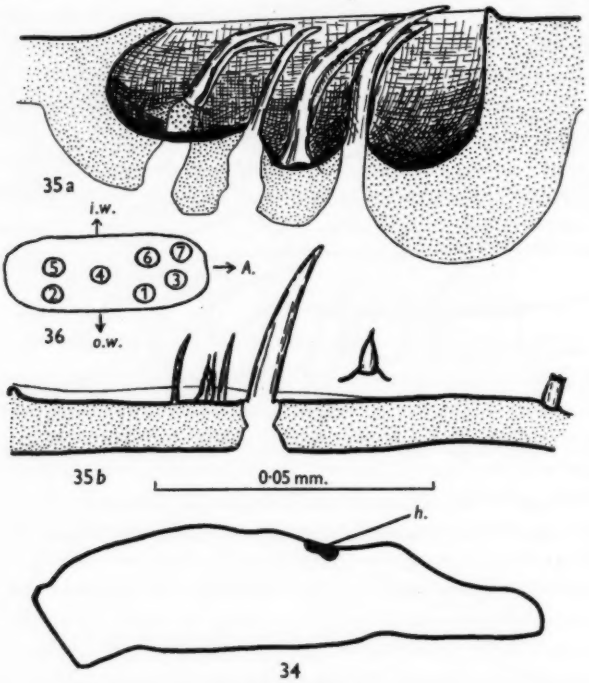
Fig. 33. Haller's organ in the nymph of *I.* (*Eschatocephalus*) *vespertilionis*.

Ixodes rothschildi (Nuttall & Warburton) 1911

The structure of this organ in the female has been figured (Fig. 8, Arthur, 1953*c*). Its concavity is slightly longer than the trough, is elongate and deeper anteriorly than posteriorly when viewed along the mid-line. It contains seven sensory hairs, which, unlike those of ticks infesting bats and of *Ceratixodes uriae*, are arranged as an anterior row of three transverse hairs, a median row of two hairs and a posterior row of two hairs. The latter are the longest and are more strongly curved than those in the front two rows. The trough is very much shallower and bears sensory hairs similar to those of *Ixodes hexagonus*. A species, *I. kohlsi* Arthur, collected from Julia Percy Island, Sir Joseph Banks Island and Robe, S. Australia, which bears a superficial similarity to *I. rothschildi* (and has been identified as such by other workers) differs in certain respects, notably in hypostomal dentition, tarsal form and very clearly in the structure of Haller's organ, where the capsule is closed and similar in its general features to *I. hexagonus*. An examination of the organ of a single female of *I. eudyptidis* Maskell shows that it conforms to that of *I. rothschildi*. This is discussed elsewhere (Arthur, 1955*a*). The nymphal form in *I. rothschildi* resembles that of the female very closely.

Ixodes unicavatus Neumann 1908

Haller's organ in the larva, nymph and female of *I. unicavatus* is located at some distance from the tip of the tarsus (Figs. 34, 39 and 40). The long axis of the concavity in the female is more or less horizontal, whilst in the nymph and larva this axis is slightly oblique. In constrast to the preceding forms described, in this species the longer and stouter sensory hairs lie in the front part of the concavity.



Figs. 34–36

Fig. 34. *I. unicavatus* female. Tarsus I to show position of Haller's organ.

Fig. 35*a*. *I. unicavatus* female. Capsule showing sensory hairs.

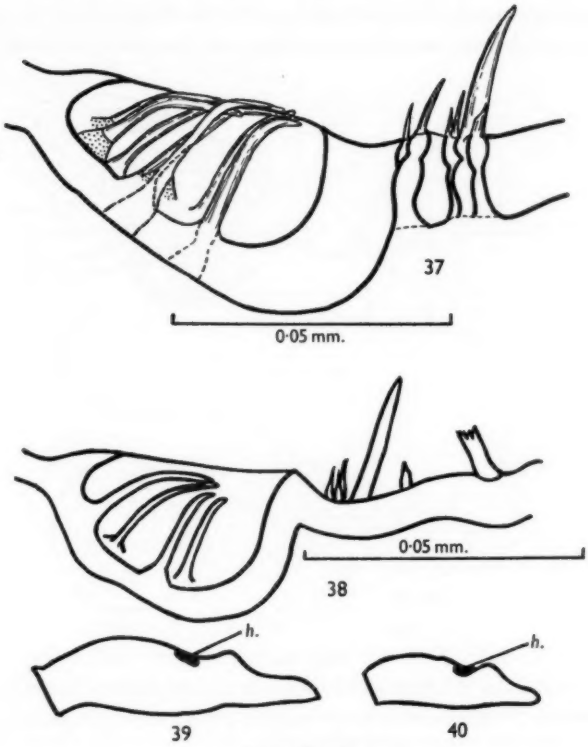
Fig. 35*b*. *I. unicavatus* female. Trough showing its shallow character and the very broad and long type IV sensory hair. Inset, type I sensillum.

Fig. 36. Plan of concavity of *I. unicavatus* showing distribution of sensilla. *A.*, anterior; *i.w.*, inner face of leg; *o.w.*, outer face of leg.

These hairs are arranged in three rows of two hairs per row with a single somewhat thinner hair placed between the second and last rows. A similar arrangement is seen in the nymph (Fig. 37), but in the larva there is an anterior transverse row succeeded more posteriorly by two hairs not transversely arranged. The trough in the female of this species is longer than the concavity, but is relatively shorter in both nymph and larva. The trough is particularly shallow, and the sensory hairs appear to arise from the upper surface of the leg very much as in *I. kerguelenensis*: it is, however, bounded by a very low wall. In all three stages the type IV sensilla is

curved, long, more than usually broad and tapers to a point (Figs. 35*b*, 37 and 38). This broad sensory hair, together with the long shallow depression, make this species easily distinguishable from *I. rothschildi*.

The capsule in other British Prostriata is oval or round. It is encased on all sides except for a small oval aperture antero-dorsally. The form of the dorsal sensory hair, in particular, and its relationship to this opening affords a means of separating these species into two major groups, viz. (*a*) those in which the dorsal



Figs. 37–40

Fig. 37. *I. unicavatus*, nymphal organ.

Fig. 38. Haller's organ in larva of *I. unicavatus*.

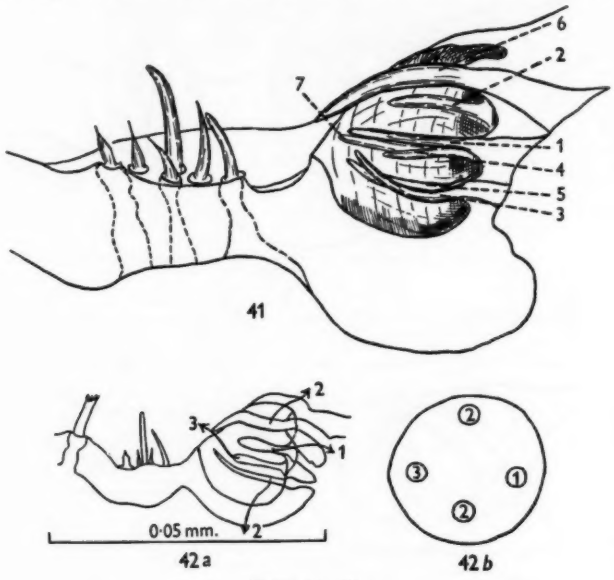
Fig. 39. Tarsus I of the nymph of *I. unicavatus* showing position of Haller's organ.

Fig. 40. Tarsus I of the larva of *I. unicavatus* showing position of Haller's organ.

sensilla protrudes through the aperture, and (*b*) those in which this sensory hair lies completely within the capsule. This separation is one of convenience rather than of fact, for one British species, viz. *I. arvicolae* Warburton, possesses a rather large antero-dorsal opening through which more than one sensory hair passes. Moreover, as far as nymphs and adults are concerned, the number of hairs within this part of the organ may be five or seven, again according to species. The larvae, however, irrespective of this number in the adult, always possess four sensory hairs in the capsule.

Ixodes frontalis Panzer, 1795

The tarsus of the fore-leg of the female possesses a slight hump before tapering gradually to the apex, and Haller's organ is situated at about two-fifths the length of the tarsus from the apex. The investing framework of the organ is thick, particularly along the floor of the capsule. Seven sensory hairs arise from the posterior wall of the capsule, and of these the downward curving dorsal sensilla, which is the stoutest and the longest, passes through the aperture and terminates in a pointed tip. The remaining hairs exhibit a diversity of form: those in the median transverse plane (1, 4, 7, Fig. 41) consist of more or less parallel-sided elements; two of these



Figs. 41, 42

Fig. 41. Haller's organ in the female of *I. frontalis* (for explanation see text).

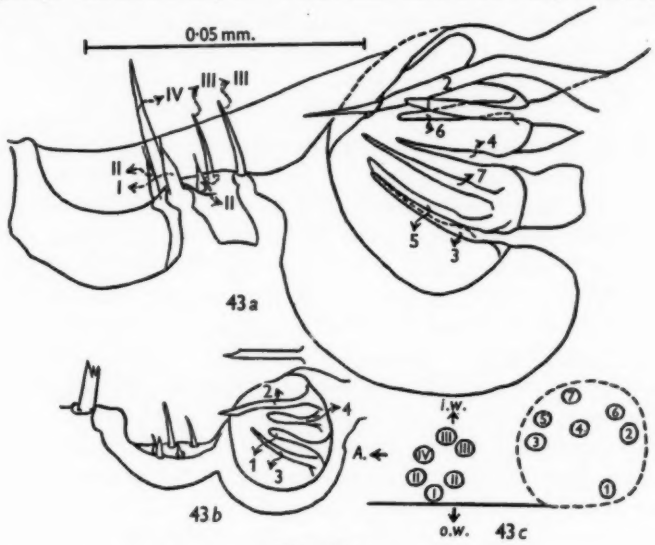
Fig. 42. *I. frontalis*. (*a*) side view of larval organ; (*b*) front view of capsule to show arrangement of sensory hairs (for explanation see text).

are about equal in length, while the intermediate hair is about half their length. Adjacent to the dorsal sensilla is a broad peg-like sensilla (Fig. 41, 2). The two ventrally placed sensory hairs appear to be more variable than the others, but one is usually thin and curved as in Fig. 41 and the other more closely resembling 1, 4 or 7. Both the anterior and posterior bristle groups consist of two long, two intermediate and two short bristles, but even the shortest bristles of the anterior group exceed the length of the longest in the posterior group. The nymphal organ is very similar to that of the adult. The major difference between Haller's organ in the larva and the female is in the number of bristles within the capsule. The dorsal sensilla (2) just emerges through the aperture and is again the stoutest element. The two lateral sensillae are club-like (1, 3), while the median ventral hair (2) is thin, long and curves up to the opening (Fig. 42*a*, *b*).

Ixodes reduvius (L.) (=*ricinus* L.)

The 'signalhaar' or dorsal sensory hair of the capsule, according to Schulze (1941), is straight with a sharply pointed tip which projects through the opening. Lees (1948) described the capsular sensillae as thin-walled, slightly curved and with blunt, rounded ends. While recognizing the 'signalhaar', he did not comment specifically on the nature of its tip.

The anterior bristle group of the female, as in *I. hexagonus*, consists of two very long and two short bristles. There are two long and four short bristles in the posterior group. Seven sensory hairs are found in the oval capsule (Fig. 43*a*); of



Figs. 43*a–c*

Fig. 43*a*. Side view of Haller's organ in *I. reduvius* female (for explanation see text).

Fig. 43*b*. Side view of Haller's organ in larva of *I. reduvius* (for explanation see text).

Fig. 43*c*. Diagram to show arrangement of sensory hairs in *I. reduvius* female. The roman numerals in the trough refer to the types of sensory hairs.

these the dorsal sensory hair is straight, thicker than the remaining hairs, parallel-sided for most of its length within the capsule, but on emerging through the aperture it narrows to a point. Sensillae 3, 4 and 7 taper towards their tips and appear to be pointed: 1, 5 and 6 are broader and appear to be rounded apically. Sensillae 3 and 5 curve upwards towards the aperture. The frame of the capsule is not as thick as that of *I. frontalis*.

Essentially Haller's organ in both the male and nymph of *I. reduvius* is similar to that of the female. The dorsal sensilla in the nymph is frequently undulate.

The larval capsule (Fig. 43*b*) is more rounded than that of the later stages. Four sensory hairs arise from the posterior wall; the dorsal sensilla may be straight or slightly undulate, and horizontal. It passes through the antero-dorsal aperture and is pointed. The curved ventral sensory hair tapers to its tip, while the two

lateral hairs are peg-like and shorter than the others. Haller's organ in ninety larvae of *I. reduvius* were examined during this survey. Of these the capsules of forty-eight contained deposits, while forty-two were free of such materials. The deposits occurred usually at the tips of the sensory hairs, were homogeneous, granular and occurred in both the tarsi of the forty-eight specimens. Deposits were found only in larvae which had recently emerged from the eggs and were lightly sclerotized; older specimens did not contain such deposits. Specimens containing such deposits were treated with xylol, alcohol and chloroform, and in no case was the deposit affected. Forty nymphs collected from the tips of grasses during their active period were also examined. No deposits were found in the organs of thirty-eight of these, but in two specimens scattered material of unknown origin was present. In no way did this material resemble the deposit found in the larval organs.

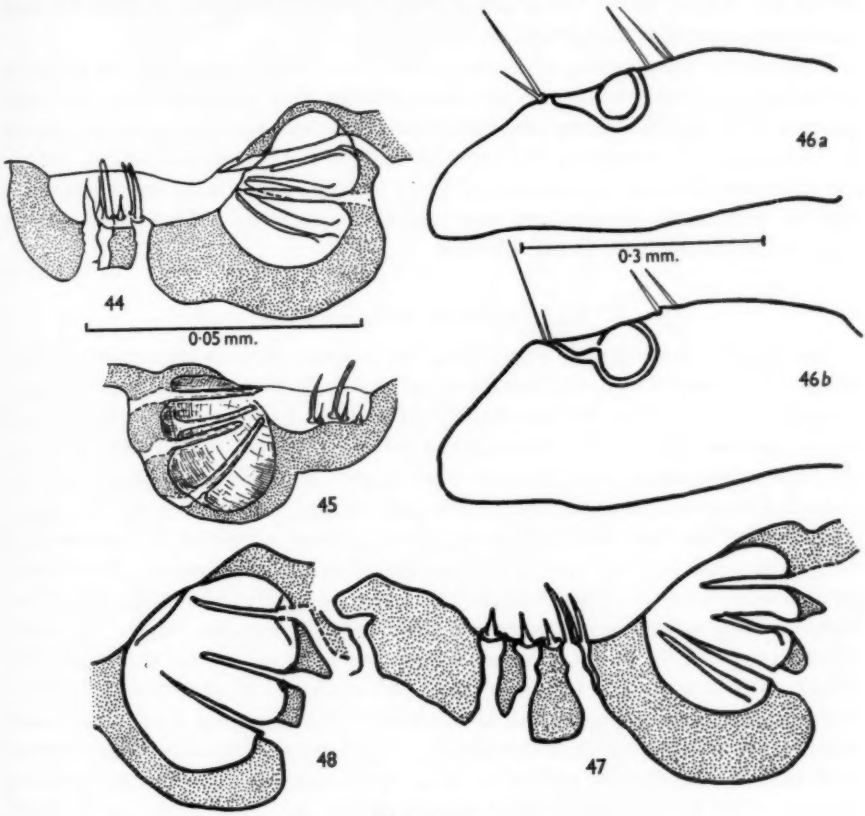
Ixodes trianguliceps Birula

Seven sensory hairs are present in the capsule of the female (Fig. 44), of which the straight dorsal hair is the longest and protrudes through the aperture and, as in *I. reduvius*, the tip is sharply pointed. The ventral hairs, which are prominently constricted basally, are as thick or even thicker than the dorsal hair and their tips come to lie just below the antero-dorsal opening. The anterior bristle group in nymphs and females consists of two long and two short bristles and the posterior group of four long and two short bristles. The capsules of the few male specimens of this species available for examination were filled with material, and in some instances a single solid oval body was present. Whether this was foreign matter or not could not be ascertained. The capsular and trough elements are similar in nymph, male and female, but in the nymph the trough is relatively shorter and bears a closer resemblance to that of the larva (Fig. 45). The anterior bristle group at this stage contains two long and two short bristles, and in the posterior group there are four bristles, about equal in length to the shorter ones of the anterior group. Four sensory hairs of approximately equal length and thickness arise within the posterior capsule and as in later stages the dorsal penetrant hair is pointed. The anterior trough has similar sensory hairs to that of the corresponding stage of *I. hexagonus*. In the larva of *I. dorrien-smithi* Turk 1945, Haller's organ is located at nearly half-way from the apex of the tarsus. It bears a close general appearance in the larval stage to that of *I. trianguliceps*; the relationship between the dorsal sensory hair and the capsular aperture could not be determined because of deposits within the capsule. The larvae of these two species can, however, be separated from one another on the form of the tarsus as shown in Fig. 46. Adult stages of *I. dorrien-smithi* were not available for examination.

Ixodes passericola P. Schl., 1933

The anterior bristle group in the female comprises two long and two shorter bristles; the posterior bristle group two long bristles, with three short bristles arranged on the corners of a triangle between their bases. The dorsal hair in the posterior capsule is parallel-sided for the most part, tapering apically, and may be straight or only very slightly curved (Fig. 47). The two lateral hairs are slightly

constricted basally, and are short, broad, pointed and lanceolate when seen in optical section. The slightly curved ventral sensillae are less constricted basally than the lateral components, narrower and drawn out to pointed tips; these are the longest sensory hairs within the capsule. Only five sensory hairs are present.



Figs. 44–48

Fig. 44. Side view of Haller's organ of *I. trianguliceps* female.
Fig. 45. Side view of Haller's organ of *I. trianguliceps* larva.
Fig. 46. Tarsus I of (*a*) *I. dorrien-smithi* and (*b*) *I. trianguliceps*.
Fig. 47. Side view of Haller's organ of female of *I. passericola*.
Fig. 48. Side view of Haller's organ of female of *I. canisuga*.

The composition of the anterior and posterior bristle groups and of the sensory hairs of the trough of the male are similar to those of the female. The ventral sensory hairs of the capsule are relatively shorter than the corresponding structures in the female. They may be straight or gently curved, while the dorsal sensory hair, as in the female, is relatively short.

The anterior bristle group of the nymph is similar to that of the male and female, but the posterior group may occasionally contain an extra small bristle, when it

resembles that of *I. hexagonus*. The dorsal and lateral sensillae of the capsule are of approximately equal length and their tips appear to be rounded when seen in optical section. The two ventral sensillae are longer than these, are more sharply tapered and bend sufficiently for their tips to come to lie beneath the aperture. The dorsal and lateral hairs, which are slightly constricted basally, frequently bear birefringent granules along their distal ends.

The anterior bristle group of the larva bears two long bristles and the posterior group has two long bristles with two short bristles between their bases. The components of the anterior trough and the posterior capsule are similar to those found in the larvae of *I. hexagonus*. The dorsal sensilla of the capsule is straight, but the exact nature of its tip is uncertain; the two lateral sensory hairs are broader and slightly constricted basally, and the ventral one narrows apically with the tip lying below the rounded dorsal aperture.

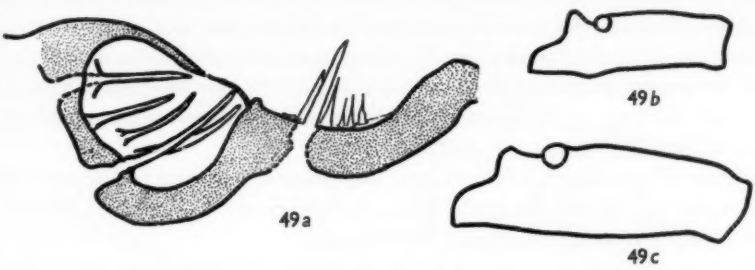
Ixodes canisuga Johnston 1849

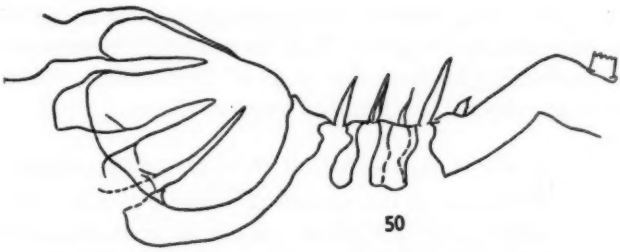
The capsule of the female organ (Fig. 48) contains five sensory hairs of fairly uniform thickness. The dorsal sensilla is straight or frequently it may have a slight upward curve, as in Fig. 48; it is slightly tapered but does not reach as far forward as the aperture and its duct through the cuticle is sharply angled relative to the long axis of the sensory hair. The two ventral hairs, both of about equal length, are at least as long as the dorsal component and usually longer. They narrow appreciably to their tips and often extend almost to the front margin of the capsule below its opening; both are very gently curved. The lateral sensory hairs are the shortest, parallel-sided for most of their length, but tapering apically and slightly constricted basally. They extend across approximately half the capsule length. A similar pattern is seen in the male organ, but the sensory hairs are stouter and longer. The trough in both male and female is short and shallow, and the sensory hairs are concentrated in the posterior half in the female. The nymphal organ agrees in all essential respects with that of the female. Four very slightly curved hairs, whose proportionate lengths are similar to those of the adults, are found in the larval capsule, while the trough elements show type III and type IV to be of similar length. Two long bristles lie in front of the anterior trough and two long and two short bristles constitute the larval posterior group.

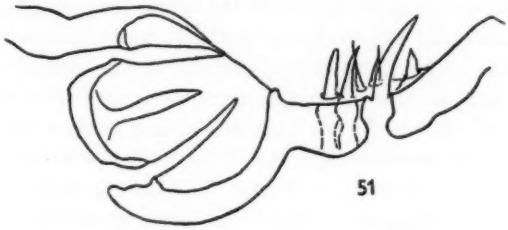
I. canisuga and *I. passericola* are difficult to separate on the characters of Haller's organ. The trough, however, does give some indication of their specific characters. In *I. passericola* it is short and obliquely placed relative to the long axis of the legs, in *I. canisuga* the trough is for the most part parallel with the long axis of the leg. The 'hump' in front of the trough in *I. canisuga* is level with the dorsal edge of the capsule, but in *I. passericola* the hump is at a corresponding higher level (cf. Fig. 49*b*, *c*).

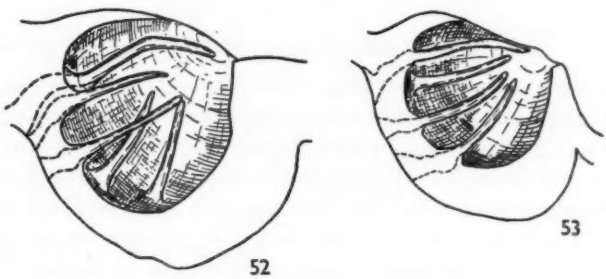
I. caledonicus Nuttall 1910

Schulze (1941) classified *I. caledonicus* into his 'sichelhaar' group on the form of the dorsal hair. This hair in the female (Fig. 52) proceeds straight for the first half of its length and then bends down to come to lie parallel with the aperture. Seven









Figs. 49–53

Fig. 49. (*a*) Side view of Haller's organ of male of *I. canisuga*. (*b*) Tarsus I of *I. passericola*, female, (*c*) tarsus I of *I. canisuga* female.

Fig. 50. Side view of Haller's organ of female of *I. baergi*.

Fig. 51. Side view of Haller's organ of male of *I. baergi*.

Fig. 52. Side view of Haller's organ of *I. caledonicus* female.

Fig. 53. Side view of Haller's organ of *I. caledonicus* larva.

sensory hairs are found in the capsule, and of these the dorsal hair is the longest, while the remaining six reach about two-thirds the distance across the capsule. The sensillae show little evidence of bending, and all appear to be morphologically similar. The sensory hairs of the trough conform to the pattern indicated in *I. hexagonus*, but the very much longer type IV sensilla frequently curves fowards. The trough is relatively short and shallow.

The anterior bristle group contains two inordinately long and two shorter bristles; the posterior group two long and two, or more rarely three, short bristles. The nymphal organ is similar to that of the female.

Four sensory hairs are present in the posterior capsule of the larva (Fig. 53). The dorsal hair does not curve as abruptly as do those of the nymph and female, but the three remaining hairs are more strongly bent than in the latter and also appear to be relatively longer (cf. Figs. 52 and 53). The anterior trough is very short and moderately deep at this stage of development. Both sets of bristle groups are supplied with two long and two short hairs.

Ixodes thompsoni Arthur 1955 (see Arthur, 1955*b*)

Tarsus I of the female of this species measures 0·51 mm. and is more or less uniform in its width to beyond Haller's organ, in front of which there is a pronounced hump before reaching the end of the tarsus (Fig. 54). The posterior capsule is oval, with its main longitudinal axis at right angles to that of the leg (Fig. 55). Seven sensilla arise within it: the dorsal element curves sharply within the capsule, tapers to its tip and passes out through the opening for only a very short distance. There is some diversity in form of the remaining sensory hairs as indicated in Fig. 55. The trough is moderately short, deep, with the six sensillae concentrated in a group about midway along its length. The trough components are similar to those of *I. hexagonus*; they are, however, distinctly broad, and the type IV sensilla narrows to a point and is always backwardly curved.

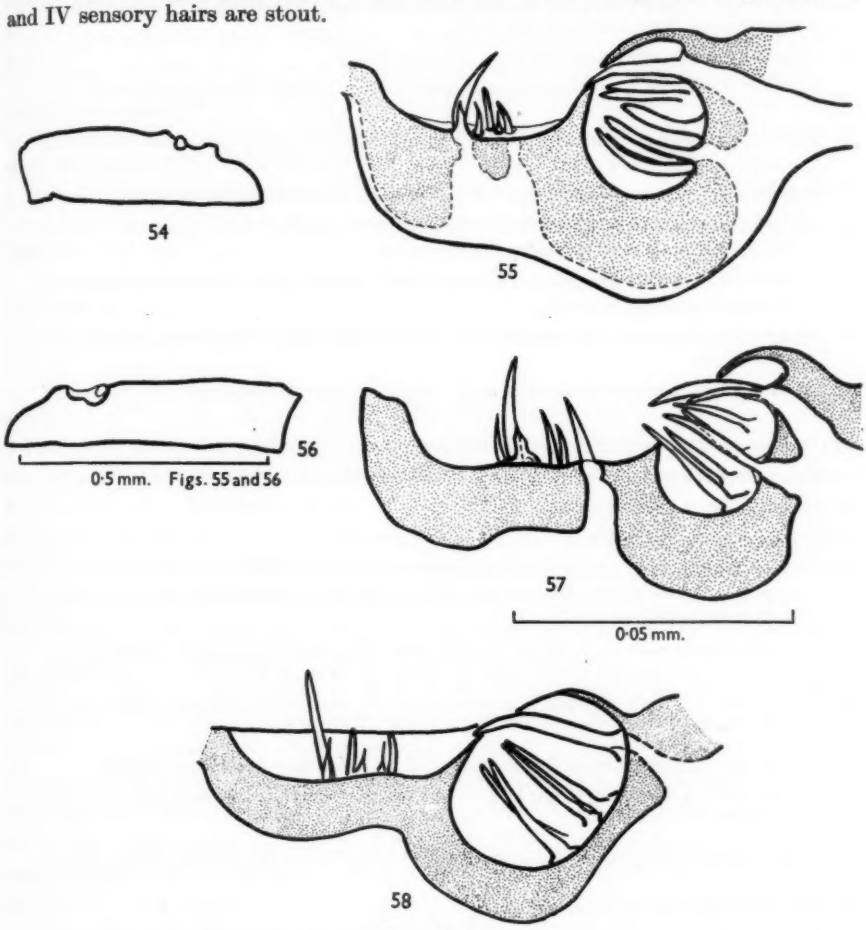
Ixodes guernseyensis Arthur 1955 (see Arthur, 1955*b*)

The capsule of this species is larger than that of *I. thompsoni*, and it differs from the latter in that the long axis is set more obliquely to that of the leg (Fig. 58). The dorsal sensory hair is straight within the capsule, bending downwards beneath the opening to pass through it for a very short distance. The other hairs show less distinction between themselves than do those of *I. thompsoni* and are more uniform in length. The trough, too, is longer and shallower than in the latter species (cf. Figs. 55 and 58), and the sensory hairs of the trough are straight. This species, the only material known, consists of females from Guernsey (Channel Islands), and was until recently diagnosed as *I. arvicolae* Warburton 1926.

Ixodes arvicolae Warburton 1926

The length of tarsus I is 0·59 mm., but unlike *I. thompsoni* it lacks a prominent hump (Fig. 56). The capsule of the female is partially sunk into the integument and behind is separated from the rest of the leg by a distinct notch. The antero-dorsal opening is wide, extending almost along the length of the capsule. Six of the

capsular sensory hairs are straight and they pass through the opening as depicted in Fig. 57. The dorsal sensory hair is broader than the others, and about equal in length to the ventral hairs. It is broad and gently curved for most of its length. The trough is long and deep with the usual forms of sensillae present; types II, III and IV sensory hairs are stout.



Figs. 54–58

Fig. 54. Tarsus I of *I. thompsoni*, female.
Fig. 55. Side view of Haller's organ of female of *I. thompsoni*.
Fig. 56. Tarsus I of *I. arvicolae*.
Fig. 57. Side view of Haller's organ of female of *I. arvicolae*.
Fig. 58. Haller's organ of female of *I. guernseyensis*. Scale as for Figs. 55 and 57.

In these British species the intraspecific variation has been determined and the morphological features of the organ in the nymph, male and female of any one British species are sufficiently constant to constitute an additional specific factor in separating the British *Ixodes* ticks. The basic character employed in distinguishing

Haller's organ in the British species depends on the form of the capsule and its contained hairs.

1. Posterior capsule open (=concavity), sensillae arising from floor 2

   Posterior capsule opening by an aperture which may be narrow or wide. Sensillae arising from floor and posterior wall. 5

2. Concavity distinctly separated from anterior trough. 3

   Concavity without a pronounced separation of the trough from the concavity. *Ixodes vespertilionis*

3. Posterior capsule sunk in trough-like depression: pronounced downward thickening between trough and concavity. *Ceratixodes uriae*

   Posterior capsule sunk in trough, without a deep intermediate thickening. 4

4. Anterior trough short, moderately deep; posterior sensory hairs of capsule thicker and longer than anterior hairs. *I. rothschildi*

   Anterior trough very long, superficial: posterior sensory hairs of capsule smaller and thinner than anterior hairs. *I. unicavatus*

5. Antero-dorsal aperture of capsule small with at most only one sensory hair (if any) protruding. 6

   Antero-dorsal aperture of capsule large, with more than one sensory hair protruding. *I. arvicolae*

6. Dorsal sensory hair protruding through aperture of capsule. 7

   Dorsal sensory hair not protruding through aperture of capsule. 11

7. Dorsal sensory hair straight. 8

   Dorsal sensory hair curved. 9

8. Ventral sensory hair not as broad as dorsal component, does not reach as far as the aperture of the capsule. Sensilla type IV of anterior trough appreciably longer than that of type III. *I. reduvius*

   Ventral sensory hair as broad or broader than the dorsal component. Sensilla type IV of anterior trough about equal in length to those of type III. *I. trianguliceps*

9. Dorsal hair (very thick when compared with the rest of the sensory hairs) curved from the base. *I. frontalis*

   Dorsal hair straight within the capsule with an accentuated bending distally. 10

10. Capsule placed vertically to the long axis of the leg: remaining sensory hairs variable in length and breadth. *I. thompsoni*

    Capsule placed obliquely to the long axis of the leg: remaining sensory hairs uniform. *I. guernseyensis*

11. Seven sensillae in posterior capsule. 12

    Five sensillae in posterior capsule. 13

12. Dorsal sensory hair straight, with a slight curve apically. *I. hexagonus*

    Dorsal sensory hair curving about midway along, so that its distal end comes to lie parallel with the aperture. *I. caledonicus*

13. Tarsus I humped. Anterior trough long, parallel to long axis. Sensory hairs of uniform pattern. *I. canisuga*

    Tarsus I with a more pronounced hump. Anterior trough short, placed obliquely to long axis of leg. Sensory hairs not of uniform pattern. *I. passericola*

In view of the similarity of Haller's organ in the nymphs and males to that of the females, the above key is equally applicable to these stages.

IX. DISCUSSION

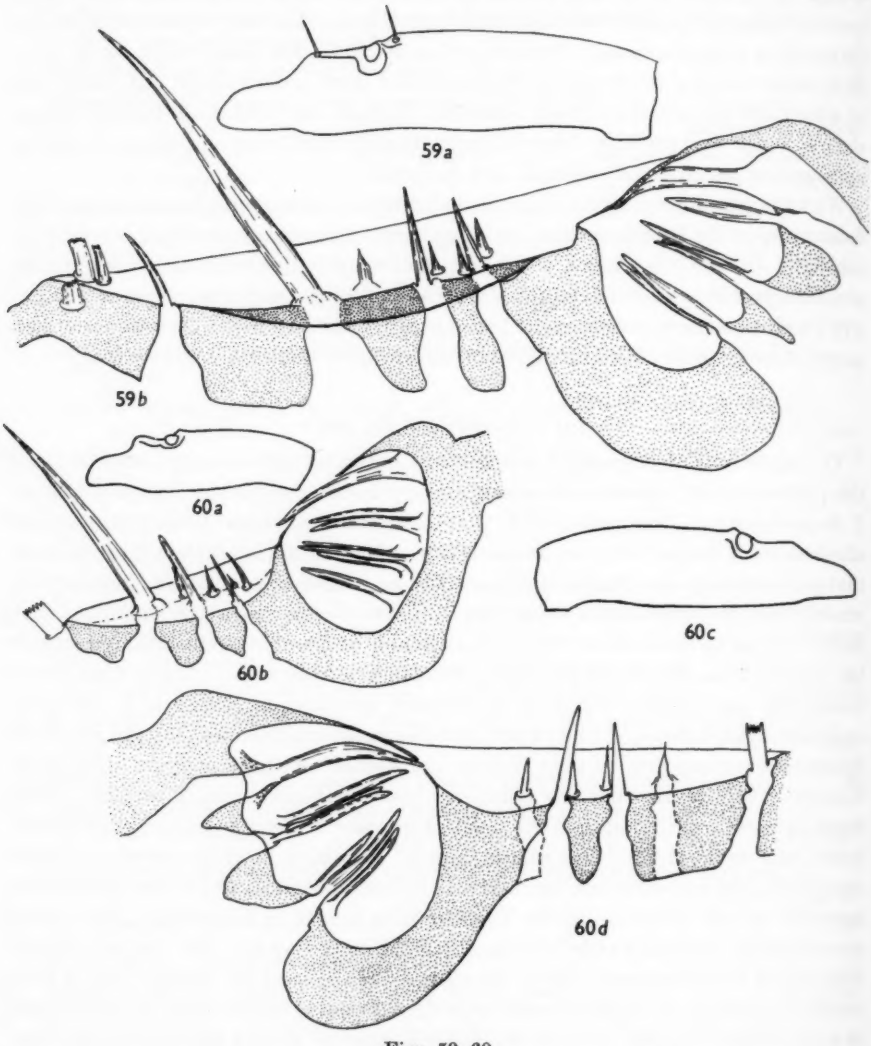
In other tick genera the relationship of the sensory hairs to the ducts within the trough differ from those seen in the genus *Ixodes*. In *Haemaphysalis* six closely bunched hairs are present; of these the longest has its own duct, while the remainder surmount a common canal. Dinnick & Zumpt (1951, MS. kindly sent by Dr Dinnick) observed that in the genus *Rhipicephalus* there are six short, stiff hairs, five of which are pin-shaped, closely bunched together, and the most distally placed sixth is longer and stronger. They all enter basally into a common duct. A similar arrangement occurs in *Hyalomma* and *Boophilus*.

With the possible exception of *Ixodes caledonicus*, intraspecific variation has been determined in the British species, and the general plan already outlined is relatively constant. On the other hand, the few specimens of individual non-British species available prohibits such a determination. Accordingly, in arriving at certain tentative conclusions here, this must be borne in mind, for on further investigation with larger supplies of material modification and amplification may be required.

(*a*) *Variability of the organ*

The organ may be superficial, without any indication of a trough or capsule, and this pattern would appear to be rare. From my investigations it is known only from *I. kerguelenensis*. Neumann (1942, 1943) and Haarløv (1943) have discussed the chaetotaxis of tarsus I in some Mesostigmata. Neumann (1942) thought it possible that an homology with Haller's organ could be established, whereas Haarløv contended that the tarsal organ in the Mesostigmata was in 'no way homologous with Haller's organ in the ticks and that consequently no proof of a relationship between the ticks and the Mesostigmata can be furnished in this way'. Both authors have considered that Haller's organ is in its most primitive condition in *I.* (*Eschatocephalus*) *vespertilionis* or a like form, and Neumann emphasizes that the groove in *Spinturnix* corresponds to the 'Wanne und kapsel' of Haller's organ. Similarly, Haarløv (1943) wrote that the homology between Haller's organ and the tarsal organ in *Spinturnix* 'hinges on the point of whether or not the groove can be interpreted as a union of the "Wanne und kapsel"'. In the light of the structure of this organ in *Ixodes kerguelenensis* no such proviso need be made, and in fact the nearest approach to this structure in the Mesostigmata is seen in *Halarachne*. This genus was originally referred to the Ixodidea by Nehring (1884), but this opinion was not maintained by Oudemans (1925). Haarløv (1943) gave as his opinion that if *Halarachne* is perhaps an original *Ixodes* type then its tarsal organ might be interpreted as a pre-Haller's organ, that is, an Haller's organ at such a primitive stage that neither the 'kapsel' nor the 'wanne' had yet been specialized. On the supposition that an 'Halarachne' type is the forerunner of the *Ixodes* then *I. kerguelenensis* provides an interesting phylogenetic link. Haarløv was unable to trace any relationship between the hair topography of *Halarachne* and the hairs of tarsus I in other Mesostigmata, with which I am in accord, but he did not comment on the possible relationships between it and Haller's organ. In his summing up on this matter he stated that it probably has no connexion with the organ of ticks.

A comparison of Fig. 26 in the present paper with figs. 3 and 4 in Haarløv's paper (1943) shows that a possible homology might exist between the sensory hairs of the two groups. There are seven hairs in an anterior group on the tarsal organ in



Figs. 59, 60

Fig. 59. Female of *I. luciae*: (*a*) Tarsus I; (*b*) Haller's organ.

Fig. 60. *I. loricatus*: (*a*) male, tarsus I; (*b*) male, Haller's organ; (*c*) female, tarsus I; (*d*) female, Haller's organ.

Halarachne which are sufficiently distinct from his figure to be classified into the categories type I, II, III and IV. In the majority of the *Ixodes* ticks only six are present, but this is no true barrier to homology for in males of *I. kerguelenensis*,

females of *I. luciae* Senevet (Fig. 59) and males of *I. loricatus* Neumann (Fig. 60*b*) seven are present, the seventh hair being long and pointed and of similar nature to the long hair of *Halarachne*. Seven hairs form the posterior group in the latter, a feature which is paralleled in most of the Prostriata, and also in *I. kerguelenensis*, but only five are present in *I. vespertilionis*; moreover, they are sickle-shaped in these ticks in contrast to the straight, pointed sensory components of *Halarachne*. To justify homologies between the Mesostigmata and the ticks requires more evidence than this, and if in fact a relationship had been established between the tarsal organ of *Halarachne* and of Haller's organ, the only common morphological features, such grounds would be tenuous in arriving at the far-reaching phylogenetic conclusions of Neumann (1942). Such a basic similarity as has been established need not be evidence in support of homology, but might even be the result of convergence.

The second generalized form of the organ possesses an open capsule (= concavity). The open capsule type of organ can be subdivided into two groups. In the first group the trough and the capsule are separated, at best, only by a narrow, transverse, low ridge and are sunk into the leg. These species are known only from bats; thus *I.* (*Eschatocephalus*) *vespertilionis* is widely distributed in Europe, Near East (Crimea and northern Iran), Japan, with the major hosts being representatives of the genera *Rhinolophus*, *Plecotus*, *Pipistrellus*, *Myotis* and *Miniopterus*.

The definitive position of *Ixodes simplex* Neumann is far from clear, but within the range of material examined there is much variability in the form of the hypostome and the palps. In all cases Haller's organ is of a uniform structure, and closely related to that of *I. vespertilionis*. This species and those close to it likewise have a wide range of distribution, and are known from the following host genera: *Rhinolophus*, *Miniopterus* and *Myotis*. *Ixodes spiculae* Arthur is known only from a male specimen from an unknown locality in South Africa.

The second group has the trough clearly differentiated from the concavity, the latter being sunk into the integument. Such organs are found in the following species: *Ceratixodes uriae*, collected from most sea birds: *Ixodes unicavatus* Neumann (1908), known from Great Britain, where the host is *Phalacrocorax carbo*; *Ixodes auritulus* Neumann 1908, a species fairly common on sea birds, widely distributed in the southern hemisphere and in the northern parts of North America. *Ixodes auritulus* has been recorded from the following hosts:

Pachyptila desolata, *Pelecanoides* sp., *Aptenodytes paragonica*. It not only lives on sea birds, but is also known from numerous and not closely related species of land birds. The following hosts are given by Cooley & Kohls (1945): *Aeronautes saxatalis*, *Passer domesticus*, Blue grouse, *Cyanocitta stelleri*, *C. stelleri carlottae*, crow, *Haliaetus leucocephalus alascanus*, fox sparrow, Junco, willow grouse, varied thrush, song sparrow, *Megalestris antarctica*, *Oreophasis* sp., *Trupialis militaris*, *Kripolegus negerrimus*, *Thamnophilus ruficapillus*, *T. caerulescens*, thrush, pipit. It is, however, not without significance that the localities from which these collections were made have a 'nautical flavour', and it is conceivable that these land birds are accidental hosts, for if their behaviour in any way resembles that of *Ixodes reduvius*, *I. canisuga* and *I. hexagonus*, they will

attach themselves to any living vertebrate above and including the reptilian stage.

I. rothschildi has been recorded from puffins, herring gull and Manx shearwater round the British coasts, and Zumpt (1952) records it off *Diomedea chlororhynchus* from Nightingale Is., *Ixodes eudyptidis* Maskell from *Eudyptula minor*, Tolaga Bay (near East Coast in North Island, New Zealand).

I. neumanni Nuttall & Warburton, 1911. This species is based on several females and nymphs from New Zealand. The host was unknown, but it is probably a sea bird and the form of the organ (Fig. 69) apparently justifies such a conclusion.

I. signatus Birula, 1895, has been recorded from *Phalacrocorax pencillatus*, *P. pelagicus*, *P. pelagicus resplendens*, from Washington, California, Alaska and Japan.

I. ornithorhynci from the duck-billed platypus in Australia.

Thus it would appear that, species from sea birds have an organ of common ground plan, but that it is not exclusive is evident from the form of the organ in *I. kerguelenensis* (from *Pachyptila desolata* and *Pelecanoides* sp.) and in *I. ornithorhynchi*.

Schulze (1941) divided the Prostriata into two groups—the 'Plynorhina' or trough-nosed and the 'Thecorina' or capsule-nosed ticks, and he contended that species hitherto in the genus *Ixodes* which have trough organs must be re-named. *Eschatocephalus* and *Ceratixodes* are already given generic status: *Ixodes collocoliae* becomes *Xiphixodes*, *Ixodes ornithorhynchi* becomes *Coxixodes* and *Ixodes unicavatus* becomes *Scaphixodes*. No mention is made of the other ticks which possess 'trough-nosed' capsules which would certainly result in the formation of a number of new genera each containing one species. The generic characters for *Scaphixodes*, for example, in addition to Haller's organ, appear weak to me—thus 'In the females (males not known) the palps, strongly curved medianly, are of equal width throughout their length; dorsal side of the collar is almost entirely occupied by one huge porose area.' If Schulze is correct in his interpretation then *Ixodes kerguelenensis* must either be classed on its own or else be included (rather dubiously) in the Plynorhina and accordingly designated generic status. This species was originally described by André & Colas-Belcour (1942) as a variety of *I. canisuga*, but Zumpt (1952) considered that it should be regarded as a new species. He enumerates the differences between *canisuga* and *kerguelenensis* thus: '*I. kerguelenensis* has a large jugular plate on each side of the pregenital plate. In *I. canisuga* these jugular plates are present, but they are very small and indistinct. Furthermore, the pregenital plate is pentagonal in *I. kerguelenensis* and more or less quadrangular in *I. canisuga*.' I have examined the type in the Paris Museum and Zumpt's specimens, and I substantiate his statements and have noted, in addition, the form of the organ. In other words, except for minor details, together with the organ structure, *I. kerguelenensis* is essentially an *Ixodes* form. Similarly, a comparison of the females of *I. rothschildi* and *I. kohlsi* show consistent differences only in the dentition of the hypostome, the form of the tarsi and of Haller's organ (for details see Arthur, 1955*a*). The hypostomal and tarsal differences are surely specific; must one then give generic rank on the form of the capsule of the organ? I feel that such a step is not justified when all the other characters are essentially those of *Ixodes*.

It should be noted here that the source of origin of the material of *I. kohlsi* from the Rocky Mountain Laboratory (RML 21545) is open to question. The legend contained within the tube of specimens reads '*I. percavatus* Neum., from *Eudyptula minor*, Julia Percy Island, Australia, 1936, J. A. Tubb'. That the specimens were taken from the host appears to be doubtful; rather it would seem that they were collected from the nesting sites of the bird, as nymphal development is very advanced. The nymphal cuticle surrounds the adult which is fully formed, except for the distension of the limbs and the straightening of the downwardly bent capitulum. The presence of males in the collection with a short, broad hypostome provided with small serrations marginally confirms this view (Nuttall, 1911). The question then arises, is this tick species a sea-bird tick or is it one which is associated with land animals primarily? Penguins are frequently parasitized by the rodent fleas *Parapsyllus* and *Listronius*, and the penguins must have acquired these from rodents. It seems, therefore, that there must have been close contact between these two vertebrate groups on the ground, and that as the penguins dispersed from the continental shores they carried these rodent parasites with them. In a similar way the same methods of transmission may have taken place in connexion with the ticks, and some support is given to this idea by the fact that the Argasid tick *Ornithodoros tajale*, which is a parasite of American rodents, occurs in the nests of penguins round the coast of South Africa as the subspecies *O. tajale capensis*. The general similarity of facies of *Ixodes rothschildi* and *I. kohlsi* suggest that they may have arisen from common stock, and that such modifications as have occurred are adaptive features. The same might also be said for *I. canisuga* and *I. kerguelenensis*, and the differences in the ventral plate formations are purely incidental changes, while the changes in the olfactory and humidity receptors within the organ may possibly be adaptive.

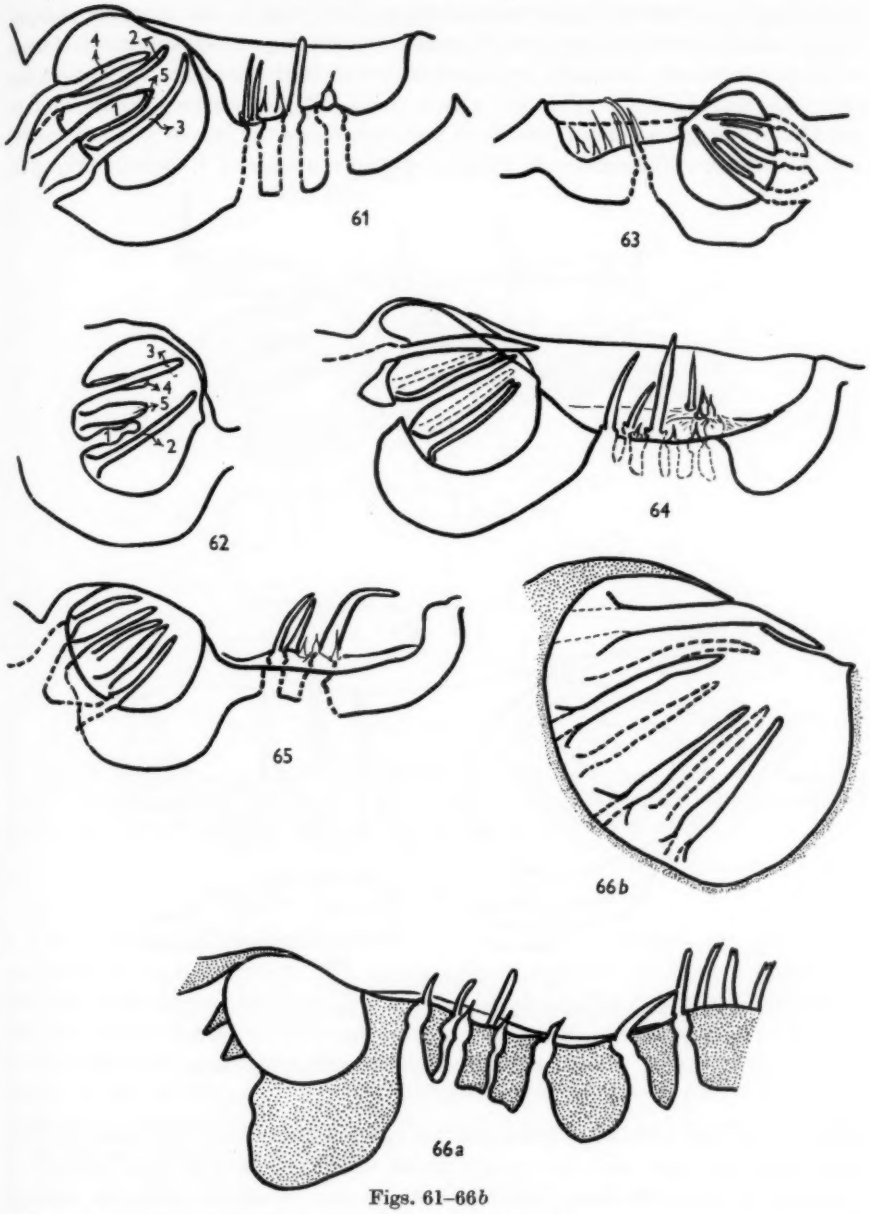
The capsules in the remaining species examined were of the closed type, and here there is considerable variation in the shape, in the inclination of the major axis of the capsule to that of the leg, and in the form and arrangement of the contained sensory hairs. A possible intermediate link between the open and closed capsules is seen in the rare *I. arvicolae* known only from Cambridge in England (Warburton, 1926), from one specimen in Denmark (Arthur, 1955*c*) and from *I. arvicolae* var. *danica* (Arthur, 1955*b*). The antero-dorsal aperture is large, and through it protrude a number of the capsular hairs. Otherwise, in closed capsuled forms one sensory hair protrudes, or they may all be enclosed.

The data for some non-British species is presented in Table 2. Within the capsule there is considerable diversity in the form of the sensory hairs; thus in *I. vespertilionis*, *I. uriae*, *I. rothschildi* and *I. unicavatus* all the hairs are of the 'sichelhaar' type, in others one hair may be of the 'sichelhaar' type (*I. caledonicus*), whilst the others may be curved or straight. In forms such as *I. reduvius* and *I. trianguliceps* no 'sichelhaars' are present. Schulze (1941) has contended that the hairs of the capsule are of fairly equal thickness and length *inter se*. This does not seem to be borne out by the present investigation, for in some species, e.g. *I. trianguliceps* and *I. reduvius*, the differences in thickness between the dorsal sensory hair and the remainder are constant and provide useful systematic characters.

These hairs, furthermore, show differences in form which are well illustrated in the American *Ixodes hearlei* Gregson (Figs. 61–63). The capsule here contains five sensory hairs. The dorsal hair is short, broad and straight, and does not reach to the aperture; lateral to it in the female is a longer, thinner sensory hair whose duct lies parallel to that of the dorsal hair. The single ventral, parallel-sided sensory hair is the longest and curves so that its tip lies against the antero-dorsal wall of the capsule. The two lateral hairs are very broad and club-like, but narrow appreciably towards their bases. A similar general arrangement is seen in the nymph and male organs of the same species. In the latter the sensory hair adjacent to the dorsal element is short and broad, while one of the lateral components is constricted basally and again at one-third of its length from the tip. Only one male was available for examination and this sensory hair may possibly be malformed. Comparable structural differences in the hairs are found, for example, in the female of *I. jellisoni*. Detailed studies also reveal that there is a considerable variation in the length of these hairs as already shown, for example, in *I. frontalis* and *I. passericola*.

The number of sensory hairs in the capsuled species may be five or seven, which is constant within the species, and of these the dorsal sensory hair usually differs morphologically from the others. Within the limits of the present data an analysis of the capsular form and its contents is of interest. Class A includes those species in which the sensory hair is straight or only slightly bent, but it does not pass through the antero-dorsal aperture. Class B is similar to class A, but the dorsal sensory hair passes through the opening. Class C has a curved dorsal sensory hair passing through the opening, the curvature might involve the whole hair or only its distal portion, e.g. *I. thompsoni* and *I. rasus*. Class D has a curved dorsal sensory hair ('sichelhaar') which does not pass through the aperture, Class E an undulate sensory hair which within the capsule may curve upwards or downwards but on emerging from it bends in the reverse direction.

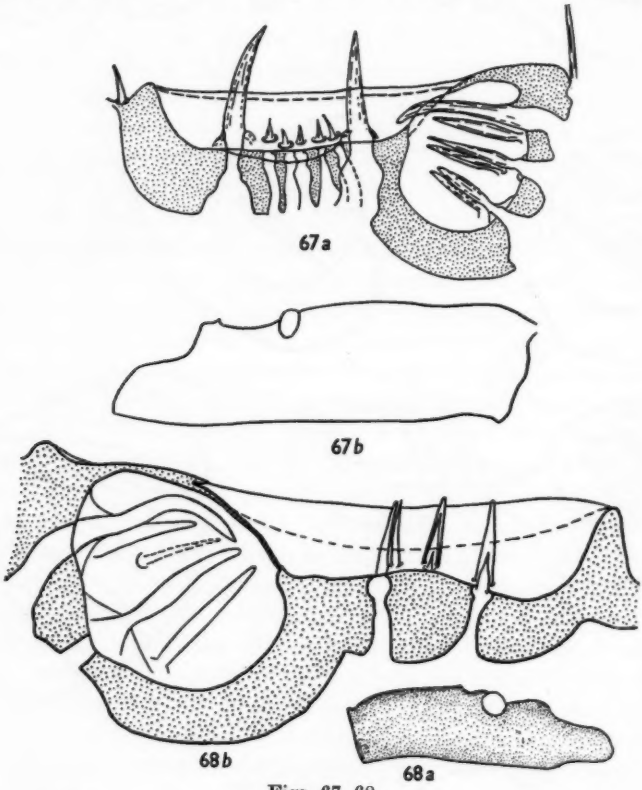
The form and number of the sensory hairs within the nymphal capsule agree substantially with that found in the adults. In the larva, on the other hand, irrespective of the number of sensory hairs in the succeeding stages, four hairs only are found—one occupies a dorsal position, another arises ventrally and two are located laterally. Usually, but not always, the dorsal sensory hair is similar in pattern to those of the adults. The anterior trough varies considerably in length and depth according to species, and at different stages of development within the same species. There is also some deviation from the 'standard' arrangement of sensory hairs as found in *I. hexagonus*, but a glance at Table 2 will show that this arrangement is very frequent through the genus. Some exceptions to this arrangement have been noted in the British species, e.g. *I. uriae*, and further examples are evident in non-British forms. In the female of *I. muris* (Fig. 64) (closed capsule) the anterior trough is long and deep and possesses a larger complement of sensory hairs than is usual in this subfamily. There are two type I, four type II, three type III and one type IV sensory hairs. In the male likewise the anterior trough is long and deep, and the sensory hairs occupy the posterior half of the trough. The sensory complement here is normal; the type IV sensilla is very broad and has a pronounced forward curve distally (Fig. 65). In the male of *I. loricatus*



Figs. 61–66*b*

Fig. 61. Side view of Haller's organ of female of *I. hearlei*.
Fig. 62. Capsule of Haller's organ of male of *I. hearlei*.
Fig. 63. Side view of Haller's organ of nymph of *I. hearlei*.
Fig. 64. Side view of Haller's organ of female of *I. muris*.
Fig. 65. Side view of Haller's organ of male of *I. muris*.
Fig. 66. Side view of Haller's organ of female of *I. acutitarsus*. (*a*) trough; (*b*) capsule.

the normal complement is supplemented by one very long, broad, tapering, sharply tipped sensilla which is placed well forward, but no comparable structure has been noted in the female. A similar arrangement occurs in the females of *I. acutitarsus* (Fig. 66) and *I. luciae* (Fig. 59) where the additional sensory component is particularly long and broad, and is at least five times the length of the type IV sensilla. Other variations in the trough occur in the male of *I. cordifer* (Fig. 67),



Figs. 67, 68

Fig. 67*a*. Haller's organ of male of *I. cordifer*.
Fig. 67*b*. Tarsus I of male of *I. cordifer*.
Fig. 68. Female of *Endopalpiger luxuriosus*. (*a*) Tarsus I; (*b*) Side view of Haller's organ.

where it has been possible to recognize one type I sensory hair, and there are four short pointed hairs and two longer broad sensillae. It has been difficult to homologize these with those found in *I. hexagonus*. A similar difficulty arises in females of *I.* (*Endopalpiger*) *luxuriosus* (Fig. 68).

An analysis of the data of available non-British ticks is presented in Table 2, where the following abbreviations are used: CAPSULE—*shape*; o, oval; r, round; e.o., elongate oval; t, triangular; no. of sens., no. of sensory hairs; *Dorsal sensilla*: s, straight; c, curved, ↓ tip directed downwards, ↑ tip directed upwards; Si, sickle-hair;

u, undulate; +, protruding through dorsal aperture; −, not protruding through dorsal aperture. TROUGH—L, long; s, short; d, deep; sh, shallow; TI, II, III, IV, different types of sensory hairs (compare with *I. hexagonus*).

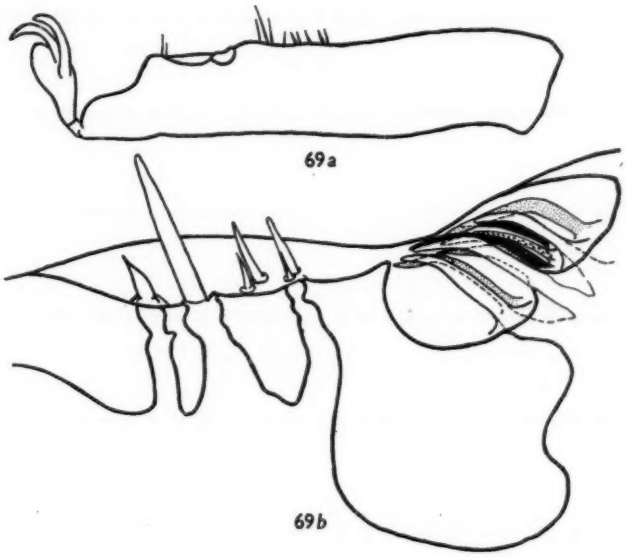


Fig. 69*a*, *b*

Fig. 69. Female of *I. neumanni*. (*a*) Tarsus I; (*b*) Side view of Haller's organ. The unshaded sensory hairs are nearest the plane of vision, then the stippled elements together with the smaller and cross-shaded hair; the more distant sensory hairs are in black.

(*b*) *Differences between the sexes*

The nymph, male and female ticks of the same species are generally alike in the contents of the trough and, size apart, I have observed distinctive sexual differences in this organ only in *I. muris* and *I. loricatus*. The normal complement of sensory hairs is found in the trough of the male of *muris*, but in the female there is a multiplication as already noted. Haller's organ in the female of *I. uriae* is similar to that of the male. The only distinguishing character appears to be a distal tarsal spur, which in the female is narrow and pointed and in the male is evident as a rounded protuberance.

(*c*) *Host relations and the form of the organ*

Even within the limits of the British material no constant correlation has been observed between the form of the organ and host selection. A much larger body of evidence is required before a generalized statement can be made, and work along these lines is proceeding.

Table 2

Species	Capsule: Stage	Capsule: Shape	Capsule: No. of sen.	Capsule: Dorsal sensilla	Trough: Length	Trough: Depth	Trough: TI	Trough: TII	Trough: TIII	Trough: TIV
I. ochotonae Gregson	N	o	7	u+	L	d	1	2	2	1
I. ochotonae Gregson	L	o	4	s+	s	d	1	2	1	1
I. bicornis Neumann	F	o	7	u+	s	d	1	2	2	1
I. soricis Gregson	N	o	7	u+	L	sh	1	2	2	1
I. soricis Gregson	F	o	7	c↑+	s	sh	1	2	2	1
I. tovari Cooley	F	r	7	c↓+	L	d	1	2	2	1
I. tovari Cooley	M	r	7	c↓+	L	d	1	2	2	1
I. spinipalpis Nuttall	M	o	7	c↓+	s	d	1	2	2	1
I. spinipalpis Nuttall	N	r	7	s+	s	d	1	2	2	1
I. pacificus Cooley & Kohls	F	o	7	c↓+	L	d	1	2	2	1
I. pacificus Cooley & Kohls	N	r	7	u+	L	d	1	2	2	1
I. pacificus Cooley & Kohls	L	r	4	u+	s	sh	1	1	2	1
I. cordifer Neumann	M	e.o.	7	c↓+	L	d	1	4		1+1(?)
I. mexicanus Cooley & Kohls	M	e.o.	7	u+	L	sh	1	2	2	1
I. dentatus Marx	F	o	7	c+	s	d	1	2	2	1
I. dentatus Marx	M	o	7	c+	s	d	1	2	2	1
I. gibbosus Schulze	M	o	7	u+	L	d	1	2	2	1
I. gibbosus Schulze	F	o	7	c+	L	d	1	2	2	1
I. angustus Neumann	F	e.o.	7	u+	s	d	1	2	2	1
I. angustus Neumann	M	e.o.	7	u+	s	d	1	2	2	1
I. angustus Neumann	N	e.o.	7	u+	s	sh	1	2	2	1
I. angustus Neumann	L	r	4	c+	s	sh	1	1	2	1
I. acutitarsus Karsch.	F	o	7	c↓+	L	sh	?1	?2	?2	?1+1
I. loricatus Neumann	M	o	7	c↓+	s	sh	1	2	2	1
I. loricatus Neumann	F	o	7	c−	L	sh	1	2	2	1+1
I. loricatus Neumann	N	e.o.	7	c−	L	sh	1	2	3	1+1
I. rugosus Bishopp	M	o	7	s−	s	d	1	2	2	1
I. rugosus Bishopp	F	o	7	s−	s	d	1	2	2	1
I. muris Bishopp & Smith	M	r	5	s+ c−	L	d	1	2	2	1

I. muris Bishopp & Smith	F	o	7	c+	L	d	2	4	3	1
I. marmotae Cooley & Kohls	M	o	7	s+	s	d	1	2	2	1
I. marmotae Cooley & Kohls	F	o	7	s+	L	d	1	2	2	1
I. sculptus Neumann	F	o	7	s−	s	d	1	2	2	1
I. jellisoni Cooley & Kohls	F	r	7	s+	L	d	1	2	2	1
I. granulatus Supino	F	e.o.	7	s+	L	d	1	2	2	1
I. granulatus Supino	M	e.o.	7	s+	L	d	1	2	2	1
I. neotomae Cooley	F	o	7	u+	L	d	1	2	2	1
I. bicornis Neumann	M	o	7	c↓+	s	d	1	2	2	1
I. affinis Neumann	F	o	7	c−	s	d	1	2	2	1
I. affinis Neumann	M	Filled with debris								
I. luxuriosus Schulze	N	o	?	?	s	d	?	?	?	?
I. luxuriosus Schulze	M	o	7	c−	L	d	1	2	2	1
I. luciae Senevet	F	o	7	c−	L	sh	1	2	2	1+1
I. sculptus Neumann	M	o	7	u−	L	d	1	2	2	1
I. pilosus Koch	M	r	7	c−	s	d	1	2	2	1
I. pilosus Koch	F	o	7	s−	s	d	1	2	2	1
I. howelli Cooley & Kohls	F	t	7	c−	s	sh	1	2	2	1
I. howelli Cooley & Kohls	M	t	7	c−	L	sh	1	2	2	1
I. howelli Cooley & Kohls	N	e.o.	7	c−	s	d	1	2	1	1
I. hearlei Gregson	F	o	5	s−	s	d	1	2	2	1
I. hearlei Gregson	M	o	5	s−	s	sh	1	2	2	1
I. kingi Bishopp	M	e.o.	7	u−	s	sh	1	2	2	1
I. kingi Bishopp	F	t	7	s↓+	s	d	1	2	2	1
I. cookei Packard	M	e.o.	7	c−	L	sh	1	2	2	1
I. spinipalpis Nuttall	F	o	7	s−	L	d	?	?	?	?
I. marxi Banks	F	r	5	s−	s	d	1	2	2	1
I. marxi Banks	M	r	5	s−	s	d	1	2	2	1
I. taiwanensis Sugimoto	M	e.o.	5	c−	s	d	1	2	2	1

Table 2 (*continued*)

Species	Capsule: Stage	Capsule: Shape	Capsule: No. of sen.	Capsule: Dorsal sensilla	Trough: Length	Trough: Depth	Trough: TI	Trough: TII	Trough: TIII	Trough: TIV
I. taiwanensis Sugimoto	F	e.o.	?	s –	s	d	1	2	2	1
I. baergi Cooley & Kohls	F	o	5	s –	s	sh	1	2	2	1
I. baergi Cooley & Kohls	M	o	5	s –	s	sh	1	2	2	1
I. texanus Banks	F	o	5	s –	s	d	1	2	2	1
I. texanus Banks	M	r	5	s –	L	d	1	2	2	1
I. ornithorhynchi (Coxixodes) Lucas	F	—	5	Si	L	sh	1	2	2	1
I. ornithorhynchi Lucas	N	—	7	Si	L	sh	1	2	2	2
I. signatus Birula	F	—	7	Si	L	sh	1	2	1	2
I. auriculus Neumann	F	e.o.	5	c –	L	d	1	2	2	1
I. japonensis Neumann	F	e.o.	7	c –	s	d	1	2	2	1
I. japonensis Neumann	M	e.o.	7	c –	s	d	1	2	2	1
I. rasus Neumann	F	e.o.	7	c↓ +	s	d	1	2	2	1
I. rasus Neumann	M	e.o.	7	c↓ +	L	—	1	2	2	2
I. rasus Neumann	N	o	7	c↓ +	s	d	1	2	2	1
I. vestitus Neumann	F	r	7	s +	L	s	1	2	2	1
I. vestitus Neumann	L	r	4	c↑ +	Very s	d	Not clear			
I. acuminatus Neumann	F	o	7	s +	s	Mod d	1	2	2	1
I. schillingsi Neumann	N	e.o.	7	s +	—	d	1	2	2	1
I. schillingsi Neumann	L	Capsule and trough filled with debris								
I. rubicundus Neumann	N	o	7	s +	s	d	Debris			
I. rubicundus Neumann	F	o	?	s	s	d	Debris			
I. ugandanus Neumann	N	e.o.?	7	c↓ +	—	d	1	2	2	1
I. ugandanus Neumann	F	r	7	c↓ +	L	—	1	2	2	1
I. neumanni Nuttall & Warburton	F	o	7	Si –	L	sh	1	2	2	1
I. boliviensis Neumann	F	o	7	c –	s	d	1	2	2	1
I. fuscipes Koch	F	o	7	s +	s	d	1	2	2	1
I. diversifossus Neumann	F	o	7	c↓ +	L	d	1	2	2	1
I. luxuriosus Schulze	F	e.o.	5	Si –	L	d	1	2	3	1+1

X. SUMMARY

1. The spiracular plate is made up of three layers, (*a*) the outermost non-porose plate, which varies in thickness, being thicker where the (*b*) intermediate layer of pedicels fuses towards the outside, and thinner over the goblets. The innermost layer (*c*) is of even thickness, and corrugated.

2. The ostium is bounded by the columella dorsally and by the lower lip ventrally, both of which are continuous with the thickened antero-ventrally placed macula.

3. The ostium opens into a subostial space which in turn passes into the atrium. The ventral and ventro-lateral walls are thrown into two folds about midway along, and then anteriorly and posteriorly merge to form a single lobe. The musculature associated with the atrium is described.

4. On morphological grounds it appears that the ostium is incapable of opening and closing, and it is suggested that only opening muscles—the dorsal and ventral atrial muscles—are present.

5. The distribution of the main tracheal trunks is described.

6. Haller's organ in the female of *I. hexagonus* consists of a trough and a capsule; the former bears six hairs divisible into types I–IV and the latter contains seven tapering sensillae. This pattern is applicable to both the male and the nymph: in the larva one type III sensilla is missing from the trough and only 4 sensory hairs are present in the capsule.

7. A description of Haller's organ in the British *Ixodes* species is given, and it is shown to be of systematic value.

8. The status of species possessing 'trough-nosed' capsules is discussed, and the validity of giving such species generic rank does not appear to be justified. As far as the material investigated is concerned there seems little difference in the sexes, with the exception of *I. muris* and *I. loricatus*, nor has any correlation been established between the structure of the organ and host specificity.

KEY TO ABBREVIATIONS

A.	anterior	*g.d.*	gut diverticulum
A.m.	muscle of ventral atrial wall	*h.*	Haller's organ
A.v.	ventral and ventro-lateral wall of atrium	*I.*	'intergoblet' cuticle formed by fusion of distal ends of pedicel
a.	atrium	*i.w.*	inner wall of Haller's organ
a.d.	dorsal wall of atrium	*L.*	lower lip of ostium
a.m.	muscle of dorsal atrial wall	*M.*	macula
B.	brick-red staining portion of cuticle	*M.T.*	median tracheal trunk
C.	columella	*M.t.*	Malpighian tubule
Ca.	capsule	*n.c.*	nerve cells
cn.	concavity	*n.f.*	nerve fibrils
D.	dorsal	*O.*	ostium
D.T.	dorsal tracheal trunk	*o.s.*	opaque tip of sensory hair
d.	distal	*o.w.*	outer wall of Haller's organ
de.	dermal duct	*P.*	posterior
E.	epimeral plate	*Pd.*	pedicel
e.p.	epithelium	*Pr.*	peritreme
g.	goblet	*p.*	proximal

KEY TO ABBREVIATIONS (*continued*)

p.v.	wall of capsule	*t.*	thin cuticle of outer plate
R.	blue staining cuticle	*tr.*	trachea
S.O.	subostial space	*V.*	ventral
S.g.	salivary gland	*V.T.*	ventral tracheal trunk
Se.	sensory hair	*V.L.*	ventro-lateral tracheal trunks
s.	stalk (of columella)	*W.*	'wedge' of macula
T.	thick cuticle of outer plate	*W.S.*	posterior wall of subostial space
T.d.	outer limit of pedicellar canal	*X.*	chitinous thickening
Tr.	trough	*y.*	aperture of capsule of Haller's organ

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(*MS. received for publication* 6. VI. 1955.—Ed.)

STUDIES ON *TRYPANOSOMA AVIUM* DANILEWSKY 1885

I. INCIDENCE IN SOME BIRDS OF HERTFORDSHIRE

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(With 2 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	308
II.	Previous work	309
III.	Materials and methods	309
IV.	Present work	311
	(i) Description of the trypanosome as seen in the blood of canaries and wild birds	311
	(ii) Description of the trypanosome as seen in cultures	314
	(iii) Identification of species	314
	(iv) Survey results	316
V.	Discussion	318
VI.	Summary	319
	References	320

I. INTRODUCTION

This paper contains a description of a trypanosome found during a survey of 297 wild birds (Corvidae) of Hertfordshire, and a discussion of its taxonomic position: it is identified as *Trypanosoma avium* Danilewsky, 1885.

My attention was first drawn to this trypanosome when an accidental discovery was made of its development in *Ornithomyia avicularia* (see Baker, 1955). Of the other two papers in this series which follow, one deals with the transmission of *T. avium* by *O. avicularia*, and the other describes the development undergone by the flagellate in avian and insectan hosts. This paper serves as a general introduction to the series, and the descriptions of methods of trapping and maintaining wild birds, of cultivating their blood, etc., will not be repeated.

It is a pleasure to acknowledge indebtedness to Professor P. C. C. Garnham, of the London School of Hygiene and Tropical Medicine, for his kindly advice and guidance throughout this work, and to Mr W. Cooper and his staff (in particular Mr R. Killick at Winches Farm) for technical assistance, and to Mr Bush and his staff for photographic work. Thanks are also given to the Library staff of the London School of Hygiene and Tropical Medicine; to Major Maxwell Knight, and Mr J. B. Yealland, Curator of Birds at Regent's Park Zoological Gardens, for much help and advice regarding the care of wild birds; to Mr Gardner, late Pests' Officer of the Hertfordshire County Council, for information as to the trapping of rooks and jackdaws in the county; and to Mr Miller, of Ayot St Lawrence, and Messrs Inns and Co., for permission to collect birds from traps which they were operating.

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II. PREVIOUS WORK

A brief account of the history of the name *T. avium* is given below (pp. 314–316).

Previous reports of trypanosomes from British birds are few. Petrie (1905) found trypanosomes in the bone-marrow (by microscopical examination) of five out of six song-thrushes and two out of six blackbirds, as well as four other species of birds, in the Elstree district of Hertfordshire. He failed to find trypanosomes in four rooks (which he referred to as 'crows') and two jackdaws. He neither described nor named these flagellates, although he published a figure of one from a blackbird. Woodcock (1910) found trypanosomes in eight out of thirty-five finches (Fringillidae) examined by blood cultures. He named this trypanosome *T. fringillinarum*. Finally, Coles (1914) reported trypanosomes from thrushes, blackbirds, and five out of six jays (*Garrulus glandarius*). He described, but did not name, these parasites.

III. MATERIALS AND METHODS

All wild birds used in the work reported in this and subsequent papers of this series were obtained in Hertfordshire. A few birds were shot or trapped at the Field Station of London School of Hygiene and Tropical Medicine at St Albans, but the majority of the rooks and jackdaws were trapped at two sites—a duck farm at Ayot St Lawrence, and a rubbish dump near Cole Green. The traps used at these two places were large wire-netting cages with an entrance in the roof consisting, usually, of a 'funnel' of wire-netting: they are described and figured on pp. 111–13 of *The Hertfordshire Farmer*, vol. 4. A few rook nestlings were obtained from their nests.

The scientific names of British wild birds used in this series of papers follows that of Witherby, Jourdain, Ticehurst & Tucker (1938–41). The approximate ages of birds are indicated by the following terminology: all birds still in the nest are referred to as nestlings; blackbirds, song-thrushes and mistle-thrushes, at the stage after they can leave the nest but before they can fly, are referred to as fledglings; rooks, after leaving the nest, are referred to as 'first-year' birds until their first moult into summer plumage, which occurs about 1 year after hatching. 'First-year' rooks can be distinguished from adults by the possession of a forwardly directed tuft of feathers at the base of the bill, which is lost in adult birds (see Witherby *et al.* 1938–41). As no such simple distinction is possible between 'first-year' and adult jackdaws, all of the latter birds which have left the nest are referred to as adults.

Birds were examined for trypanosome infections either by means of cultures of their blood and/or bone-marrow on N.N.N. medium, or by examination of blood films (fresh, or dried and stained). The N.N.N. medium was prepared by a modification of the method described by Wenyon (1926, pp. 1300–3), using 6 g. of granulated agar and 3 g. sodium chloride to every 500 c.c. water. When distributed in 3 c.c. amounts in test-tubes, 30 drops of fresh rabbit's blood were added to each tube. The amount of bird's blood used to inoculate these tubes varied, but was usually between 0·1 and 0·5 c.c. Some attempts to cultivate larger volumes of

blood (obtained from rooks and jackdaws by cardiac puncture, as described below) on nutrient broth failed, because blood obtained in this way frequently became contaminated. Culture tubes were incubated at 30° C., and examined usually twice during the next 10 days. Tubes not showing flagellates at the end of this period were considered negative. Often two or three tubes were inoculated with one sample of blood. In all reports of the number of N.N.N. tubes inoculated with a bird's blood given in these papers, tubes which became contaminated with bacteria or fungi are ignored (unless flagellates developed in them also, which happened only rarely).

Cultures of strains of trypanosomes isolated from a jackdaw and an experimentally infected canary are being maintained in this laboratory on N.N.N. medium incubated at 30° C., fresh tubes being subinoculated weekly. At the time of writing (April 1955) these strains have been maintained for about seventy generations.

For purposes of description and illustration, some cultures were grown upon N.N.N. medium in Petri dishes (see Wenyon, 1926, p. 1304). Preparations were made from these 'plates' by laying a clean cover-slip over the area of surface on which flagellates were growing, and then removing the cover-slip, fixing, staining, dehydrating and mounting the film on it without allowing it to dry (see Qadri, 1952). The precise treatment of these preparations was the same as that used in preparing slides of flagellates from the gut of insects, and will be described in detail in the third paper of this series. Routine dried blood films were stained with Giemsa's stain. The measurements of trypanosomes given in Table 1 were made from outline camera lucida drawings of specimens from these dried preparations. As the flagellates were usually somewhat convoluted, measurements were made, when necessary, by means of a small measuring-wheel on a threaded axle, such as is used for measuring distances on maps. Fresh blood preparations were examined under the $\frac{2}{3}$ in. objective, the large trypanosome being seen easily.

Bone-marrow for cultivation or microscopic examination was obtained (at autopsy only) by a modification of the method described by Diamond & Herman (1954). The bird's leg was severed with strong scissors just below the head of the tibio-tarsus, after removal of feathers from this region. The muscles were then removed, and the severed end of the bone dipped quickly into 70% alcohol and flamed. Marrow was then removed from the tibio-tarsus with a sterile Pasteur pipette containing a little sterile saline (0·85%), and expelled (with aseptic precautions) into a tube of N.N.N. culture medium, which was then incubated at 30° C. and examined as for a blood culture (see above).

Cardiac punctures, both for inoculations and for removal of blood, were performed on rooks and jackdaws. A fairly large needle was used (gauge 1 for rooks, gauge 12 for jackdaws), and was inserted vertically through the sternum about midway along its length and some 5 mm. to the left of the keel. These birds tolerated the operation well. It was performed under light Nembutal-induced anaesthesia, the dose of Nembutal given being usually about 0·2 c.c. to rooks (weighing about 425 g.), and 0·15 c.c. to jackdaws (weighing about 175 g.): the tolerance of these birds to Nembutal varied considerably, however. It was found

impossible to induce deep anaesthesia with Nembutal alone, without administering a lethal dose.

During the course of this work, many wild birds were kept in aviaries for periods of up to 2 years. Blackbirds and thrushes were fed on a proprietary insectile mixture such as 'Cecto' (made by Messrs Trower and Co., of London) and fruit (e.g. apples). Rooks and jackdaws were fed on rolled or crushed oats and minced raw meat. Nestling rooks were reared in the laboratory in a hay-lined box, and were fed frequently by hand on boiled meat, boiled meal-worms (larvae of *Tenebrio molitor*), boiled hens' eggs, and 'Cecto'.

IV. PRESENT WORK

(i) *Description of the trypanosome as seen in the blood of canaries and wild birds*

In fresh preparations of blood the flagellate appears as a large, spindle-shaped body. The flagellum is active, but the organism itself moves only slowly through the plasma, with the flagellum directed forwards. The only internal structure visible by transmitted light is the nucleus which appears oval or circular with a central, darker, circular body enclosed within. The kinetoplast cannot be seen. The body tapers to a fine point at both ends: the anterior end, which follows the flagellum to within about 5 or 10 μ of the latter's end (see Table 1), is supple, moving about as the flagellum lashes. The posterior end, on the contrary, is quite stiff, and usually has a slight but consistent curve; it is drawn out until it becomes almost as fine as the flagellum.

Under phase-contrast illumination, the appearance of the flagellate is very similar to that described above. The nucleus is the only inclusion that can be seen (with the exception of a few small granules near the posterior end of one specimen). The nuclear structure appears as described above, but anterior and posterior to the nucleus, and immediately next to it, two more-or-less triangular structures can be seen. These were present in all of the three specimens from a canary examined under phase-contrast. In one, the structure appeared to consist of three granules at the apices of a triangular, clearer region, the granules being joined by a line. The existence of some structure in this region is confirmed by the presence of two lighter coloured areas there, in stained preparations.

In dry-fixed blood films, stained with Giemsa's stain, the general appearance of the parasite is as described above. The cytoplasm stains a deep, usually homogeneous, blue. Longitudinal striations can be seen in the majority of specimens. The red-staining flagellum may run along one side of the body, or it may cross it once or twice. The nucleus appears as a solid red-stained mass and is circular, oval, or even nearly triangular. When elongated it lies usually either at right angles to the long axis of the trypanosome, or obliquely to it. The kinetoplast is prominent and stains deep red. Adjacent, and anterior and posterior, to the nucleus are the two areas referred to above. These (which can be seen in the great majority of specimens) stain a pale blue and are roughly triangular in shape: they do not appear to have definite margins. The undulating membrane is conspicuous but does not stain: where it crosses the body it is represented by a lighter coloured band.

In preparations of blood fixed while wet in osmic 'acid' vapour followed by ethanol, and subsequently stained with Giemsa and mounted without being allowed to dry, the structure of the trypanosome appears identical to that already described. The measurements given in Table 1 are taken from dried preparations, since it was felt that these were more comparable with those of most previous descriptions. Some typical 'blood-type' trypanosomes, as described, are shown in Fig. 1. Table 1 contains the average measurements of twenty-five specimens from a 3-day-old experimental infection in a canary, together with those of five specimens from an infection in a blackbird, three specimens from two rooks, and a single, poorly stained specimen from a jackdaw. The blackbird (*Turdus merula merula* L.) was found to be infected after it had been in captivity for some months,

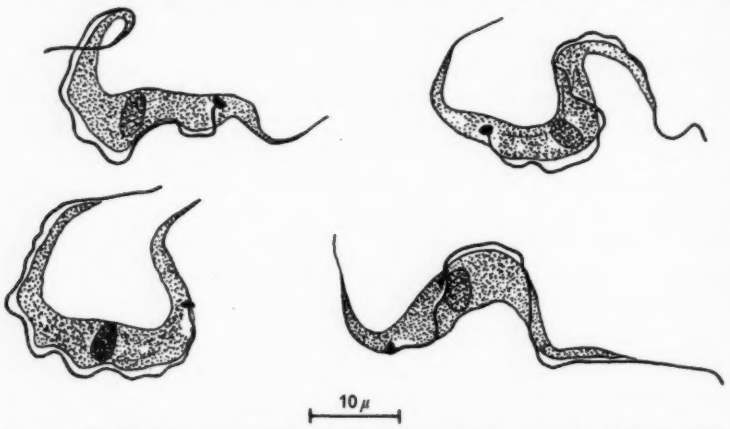

Fig. 1. Camera lucida drawings of *T. avium* from dried, Giemsa-stained films of peripheral blood. Top row: two trypanosomes from a 3-day-old experimental infection in a canary. Bottom row: two trypanosomes from a blackbird.

and so may not represent a strictly natural infection. The slightly larger average size of the trypanosomes from this blackbird, as compared with those from the canary, is presumably due to the fact that the blackbird's infection was of longer standing: some of the flagellates from the canary are therefore probably not quite fully grown. A comparison of the figures shows that the difference in size is not great and is restricted to the body length and total flagellar length. The modal value of the body length of the trypanosomes from the canary is 49·5–50·9 μ, which approaches quite closely to the average value for the length of the blackbird trypanosomes, 52·1 μ. Whether the shorter average length of the rook trypanosomes is due to the same factor is not known. The last column of Table 1 contains the grand averages of all the flagellates measured. This was calculated by dividing the grand total of each dimension by the number of flagellates measured, and is inserted in the table to facilitate comparison with measurements of other avian trypanosomes.

The description of stained trypanosomes given above is based on the appearance of parasites from canaries, a blackbird, rooks and a jackdaw. In all naturally

Table 1. *Measurements in microns of* Trypanosoma avium *from dried films of the blood of various birds*

Trypanosomes from ...	Canary (3-day-old experimental infection)		Blackbird		Rooks (two)		Jackdaw	Grand average
	Average	Range	Average	Range	Average	Range		
Length of body (excluding free flagellum)	47·7	34·9–54·3	52·1	48·2–60·3	43·7	35·0–57·5	54·7	48·2
Length of free flagellum	7·2	3·7–12·7	6·2	4·5– 7·0	7·4*	4·5–10·2	—†	7·1‡
Total length of flagellum	52·9	45·3–63·0	56·0	51·6–62·0	57·4*	54·0–60·7	—†	53·7‡
Posterior end to centre of kinetoplast	14·6	7·8–19·8	14·7	11·5–20·2	10·0	4·7–20·0	11·8	14·1
Posterior end to centre of nucleus	24·6	15·2–30·0	24·9	21·5–30·0	20·0	15·7–27·3	24·6	24·2
Nucleus (opposite diameters)	4·2 × 2·4	3·0–5·5 × 1·5–3·0	4·9 × 3·0	4·0–6·0 × 2·8–3·5	4·1 × 2·7	3·3–5·0 × 2·3–3·3	5·0 × 2·3	4·4 × 2·5
Kinetoplast (opposite diameters)	1·1 × 0·7	0·5–2·0 × 0·5–1·0	1·0 × 0·8	0·8–1·5 × 0·5–1·0	1·0 × 0·8	0·5–1·5 × 0·5–1·0	1·5 × 0·8	0·7 × 0·5
Breadth at level of nucleus (excluding undulating membrane)	5·7	3·5– 9·0	5·5	5·0– 6·0	4·6	3·5– 6·0	4·5	5·5
Maximum breadth of undulating membrane	1·3	0·8– 2·0	1·4	1·0– 1·8	1·5	1·3– 2·0	—†	1·4§
Number of trypanosomes measured	25		5		3		1	34

* Measurable in only two specimens.
† Flagellum not stained.
‡ Measurable in only thirty-two specimens.
§ Measurable in only thirty-three specimens.

infected birds, parasitaemia was so low that the parasites found in stained smears were too scanty for careful comparisons to be made, as can be seen from Table 1, where the numbers of rook, jackdaw and blackbird trypanosomes measured are equal to the total number of undamaged specimens found on stained films. Several other trypanosomes were seen in fresh preparations of blood from these birds and from an experimentally infected mistle-thrush (*Turdus viscivorus viscivorus* L.) and closely resembled one another. All these trypanosomes are therefore assumed to belong to the same species, since not only are they almost identical in appearance but, as will be described later in this and succeeding papers, they have been transmitted (naturally or artificially) from rooks and jackdaws to canaries and a mistle-thrush, as well as to other rooks.

(ii) *Description of the trypanosome as seen in cultures*

The cultures referred to below were grown on N.N.N. blood-agar medium at 30° C., either in tubes or on plates.

Crithidial forms were predominant: a great variety of these was present, ranging from large, broad forms to very long, narrow individuals. Many were seen to be undergoing division—almost invariably by longitudinal binary fission. However, in some of the cultures grown on plates of N.N.N. medium, a few large masses of cytoplasm were seen: these masses were of irregular shape and contained several nuclei and kinetoplasts, each of the latter with an attendant flagellum. A few rounded forms, presumably involution stages, were also seen in most cultures. Metacyclic trypanosomes (which will be described in a later paper) were not seen in any cultures. Large forms with the trypanosome arrangement of nucleus and kinetoplast were, however, seen in several tubes, usually in small numbers. Their general shape was reminiscent of the 'blood-type' trypanosomes described above, but they were somewhat smaller and their nuclei were more posterior in position and consequently nearer to the kinetoplast. The significance of these forms is obscure. Typical forms from cultures and some of these trypanosome forms are shown in Fig. 2.

(iii) *Identification of species*

The trypanosome which is described in this paper resembles quite closely that described by Danilewsky (1889) as '*T. avium majus*'. This, together with '*T. avium minus*', was found in owls (species not given) and roller-birds (*Coracias garrulus*). Danilewsky stressed that the presence of intermediates between the '*majus*' and the '*minus*' forms showed that both belonged to the same species. This species he referred to by several descriptive names, and from this paper (1889) alone it is not clear which he intended as the specific name of the trypanosome. In an earlier paper (Danilewsky, 1885), containing the first description of the parasite, he referred to it as *T. avium* only. It seems to me, therefore, that *T. avium* is the correct name for this parasite. Coatney & Roudabush (1937) and Coatney & West (1938) have also regarded it as correct. Laveran (1903) proposed to restrict *T. avium* to the trypanosome found in wood owls (*Syrnium aluco* = *Strix aluco*). Novy & MacNeal (1905) described as *T. avium* Novy & MacNeal emend. a

dimorphic trypanosome which they found in several species of birds, including members of the families Corvidae and Turdidae. A 'large' and a 'small' form of this trypanosome were described, the large form resembling that described by me.

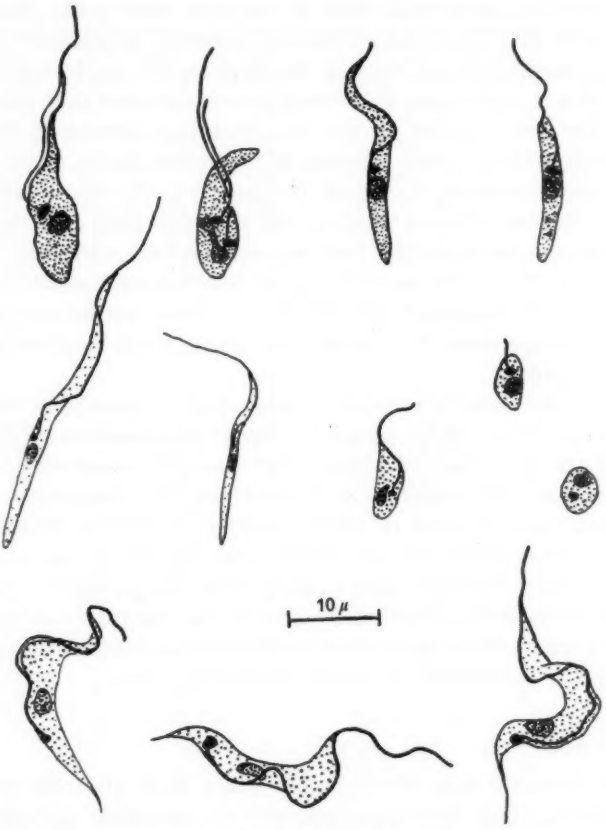

Fig. 2. Camera lucida drawings of cultural forms of *T. avium*. Top row: forms from wet-fixed preparations made from a 4-day-old plate culture on N.N.N. medium. Middle row: forms from wet-fixed preparations made from a 7-day-old plate culture on N.N.N. medium. Bottom row: three trypanosome forms from a dried, Giemsa-stained, preparation made from various pooled cultures.

Because of the wide host-specificity of most avian trypanosomes, including the present one, which have been studied from this viewpoint, there seems to be no basis for Laveran's (1903) restriction of the name *T. avium*, and consequently no need for Lühe's (1906) new name, *T. confusum*, to contain Novy & MacNeal's flagellate.

The only other trypanosome from birds of the families Corvidae and Turdidae to receive a name is *T. corvi* of Stephens & Christophers (1908), found in the Indian crow (*Corvus splendens*). From its very brief description, this trypanosome

could be the same as that described in the present paper, in which case its name should be regarded as a synonym of *T. avium*.

Unless the 'small' form (of Danilewsky and Novy & MacNeal) is represented by the growth stages of the present trypanosome (which will be described in a later paper), nothing comparable with it has been seen in the present survey. However, it is felt that this is not a sufficient reason for the adoption of a different specific name, particularly as Novy & MacNeal report the finding of one form without the other in some cases: they based their assumption that these two forms belonged to the same species on the fact that they developed identically in cultures. Whether this is a valid criterion is little more than a matter of opinion, and it may prove necessary to regard Danilewsky's '*T. avium majus*' and '*T. avium minus*' as two different species, but it is felt that there is insufficient evidence at the moment to render this necessary or even advisable. The cultural development described in the previous section does not agree absolutely with that described by Novy & MacNeal (1905) for their *T. avium* but as their cultures were incubated at a temperature 5° C. lower than mine, much significance cannot be attached to this point.

In the case of organisms as variable in morphology as these large trypanosomes, too much reliance must not be placed on sets of measurements of dried, stained specimens for the separation of species. For example, considering only the five flagellates from an 'old' infection in a blackbird, the measurements of length (excluding flagellum) recorded in Table 1 vary from 48·2 to 60·3 μ, a range of 23% of the average value (52·1 μ). With such organisms, host-specificity and cross-immunity tests should be used to supplement morphological characteristics in the definition of species. Until these are done for several avian trypanosomes, it seems best to place this trypanosome in the first described species, with which it is morphologically compatible, *T. avium* Danilewsky, 1885.

(iv) *Survey results*

During the 9 months from March to November 1954, 216 rooks (*Corvus frugilegus frugilegus* L.) and sixty-nine jackdaws (*C. monedula spermologus* Vieill.) were examined by means of cultures inoculated with their peripheral blood. Eleven rooks and one jackdaw were examined by cultivation of their bone-marrow only. The results of this survey are shown in Table 2. They can be summarized as follows:

(1) One hundred and sixteen adult rooks (i.e. those hatched in or before 1953) were examined; twenty-six of these (22·4%) were found to be infected with trypanosomes.

(2) One hundred and eleven 'first-year' rooks (hatched in the spring of 1954) were examined; only one was found to be infected (on 22 August).

(3) Seventy jackdaws were also examined; six (8·6%) were found to be infected.

In interpreting these results, the following facts should be taken into account:

(i) The number of 'first-year' birds examined reached its peak in June, after which the numbers which were trapped declined rapidly; the first infected

Table 2. *Numbers of birds examined for* Trypanosoma avium, *and those found to be infected, in* 1954

1954 Week commencing	First-year rooks (hatched in 1954)			Adult rooks (hatched 1953 or before)			Jackdaws (any age)		
	Number examined*	Number infected	Percentage infected	Number examined*	Number infected	Percentage infected	Number examined*	Number infected	Percentage infected
7 Mar.	—	—	—	11	0	0	1	0	0
14	—	—	—	10	0	0	6	0	0
21	—	—	—	4	0	0	3	0	0
28	—	—	—	9	0	0	8	0	0
4 Apr.	—	—	—	4	0	0	4	0	0
11	—	—	—	—	—	—	—†	—	—
18	4	0	0	7	2	28·6	2	0	0
25	6	0	0	5	2	40·0	4	2	50·0
2 May	1	0	0	2	0	0	1	0	0
9	3	0	0	5	1	20·0	5	1	20·0
16	1	0	0	9	2	22·2	5	0	0
23	8‡	0	0	10	3	30·0	2	0	0
30	3§	0	0	9	2	22·2	—	—	—
6 June	12	0	0	1	1	100·0	—	—	—
13	11§	0	0	1	1	100·0	1‡	0	0
20	4	0	0	7	4	57·1	—	—	—
27	6	0	0	6	3	50·0	—	—	—
4 July	5	0	0	6	0	0	—	—	—
11	9	0	0	1	0	0	—	—	—
18	10	0	0	—	—	—	—	—	—
25	4	0	0	4	1	25·0	—	—	—
1 Aug.	3	0	0	2	1	50·0	—	—	—
8	6	0	0	1	1	100·0	—	—	—
15	7	0	0	1	1	100·0	—	—	—
22	2	1	50·0	—	—	—	—	—	—
29	1	0	0	1	1	100·0	5	1	20·0
5 Sept.	1	0	0	—	—	—	—	—	—
12	—	—	—	—	—	—	7	1	14·3
19	2	0	0	—	—	—	—	—	—
26	1	0	0	—	—	—	—	—	—
3 Oct.	—‖	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	7	1	14·3
17	—	—	—	—	—	—	9	0	0
24	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—
7 Nov.	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
28	1	0	0	—	—	—	—	—	—
Totals	111	1	0·9	116	26	22·4	70	6	8·6

* All birds examined by blood cultures on N.N.N. medium, unless otherwise stated.

† Broth cultures made from five jackdaws, but all contaminated.

‡ Examined by bone marrow cultures only.

§ Including two (w/c. 30 May) and one (w/c. 13 June) examined by bone marrow cultures only.

‖ One bird examined, but culture contaminated.

'first-year' bird was found in late August. As the trypanosome is transmitted by *Ornithomyia avicularia*, which is active from late May to November only (Baker, 1955), the percentage of infected 'first-year' birds would be expected to rise just at the time that the catches of them in the traps declined. Thus it is probable that the figure of 0·9% infected 'first-year' birds is too low for the year as a whole, since if more birds had been caught in late August, September and October, it seems likely that more infected individuals would have been found.

(ii) No birds were shown to be infected by peripheral blood cultures until the middle of April. It is my belief (for which evidence will be given in a later paper of this series) that this is due to a seasonal disappearance of the trypanosome from the peripheral circulation during the winter. If this is so, a truer picture of the number of infected rooks and jackdaws would be obtained by ignoring the birds examined before, say, 18 April. If this is done, the figures for the numbers of birds infected become: adult rooks, 78 examined, 26 (33·3%) infected; jackdaws, 48 examined, 6 (12·5%) infected.

(iii) At first sight, the infection rate for rooks and jackdaws seems to differ greatly. However, as pointed out above (p. 309) it is not possible to tell the difference between 'first-year' and adult jackdaws easily, and so no attempt was made to classify jackdaws in this way. Thus the figures for jackdaws in Table 2 should be compared with those for 'first-year' and adult rooks combined. If the figures for rooks of both groups are so combined, the following result is obtained: number of rooks of both age groups examined after 18 April, 189; number found to be infected, 27; percentage infected, 14·3. This figure of 14·3% corresponds quite closely to that obtained for jackdaws during the same period, which was 12·5%. It seems probable therefore that the proportion of the adult rook population infected (i.e. one-third) which was deduced above, may be applied also to the adult jackdaw population.

Diamond & Herman (1954), working with Canada geese (*Branta canadensis* L.), found that cultures inoculated with bone-marrow from living birds gave a much higher figure for the percentage of infected geese than did cultures inoculated with heart blood from the same birds (40·2% infected as against 3·9%). A small trial was conducted to see if this method of culturing would also be a more reliable guide in the case of rooks. In this trial, however, bone-marrow collected at autopsy was used. Of twelve adult rooks both blood and bone-marrow cultures of which were uncontaminated, flagellates developed in the blood cultures of nine, but in the marrow cultures of six only. It should be borne in mind that the latter trial was carried out in June, whereas Diamond & Herman's work was done in April. It is possible that the trypanosome of the Canada goose is restricted more or less entirely to the bone-marrow at that time of the year, as it is in rooks and jackdaws of this country in winter and spring.

V. DISCUSSION

The identification of this trypanosome found in English rooks and jackdaws as *Trypanosoma avium* Danilewsky 1885, is the first occasion on which a trypanosome of corvid birds in this country has been assigned to a particular species. It is

interesting to note that this species appears to have a fairly 'loose' host-specificity, since in the work described in this and subsequent papers it has been found in, or introduced into, rooks, jackdaws, canaries, a mistle-thrush and a blackbird. In view of this wide host-range, it is possible that some of the trypanosomes described from other British birds will be found to belong to the same species. Petrie (1905) did not describe those which he found in blackbirds, thrushes and other birds, but he figured one form from a blackbird which does not much resemble the trypanosome described in this paper. Woodcock's (1910) *T. fringillinarum* was very polymorphic: some of the forms he found in red-polls (*Carduelis* = *Linota rufescens*) in autumn resemble the trypanosome described by me. Birds of the family Fringillidae (from which Woodcock's species was described) were not examined in the survey reported above (pp. 316–318). Until more is known of the host-specificity of these parasites, it is impossible to say whether or not *T. fringillinarum* is a distinct species. It seems likely that the trypanosomes described by Coles (1914) from English mistle-thrushes, blackbirds and jays belong to the same species as that which was found in the present survey. The forms Coles described from the jays (fam. Corvidae) and some of the forms he saw in the mistle-thrush resemble the '*T. avium majus*' type. Some of the thrush flagellates, and all those from the blackbirds, were, however, rather smaller.

It may be concluded, from the results and interpretation of the survey reported above (pp. 316–318), that about one-third of the adult rook population in this district of Hertfordshire, and probably about the same proportion of adult jackdaws, were infected with trypanosomes at the time this survey was made. The overall figure for the percentage of birds infected, including rooks and jackdaws of both age groups, was about 11 % (33/297). There are no surveys of British corvids with which these figures can be compared. Woodcock (1910) found nearly 23 % (8/35) finches infected: his sample presumably consisted of birds of all ages. Coles (1914) found five out of six jays infected, but this sample is not large enough to warrant comparison.

The results reported in the present paper suggest that trypanosome surveys of British birds should be carried out during the summer months (say May to October) in order to gain the truest picture of the number of birds infected. Also, such surveys should not rely only on the examination of blood or even bone-marrow preparations, but should also be conducted by making blood cultures. It seems that, during the summer months, no advantage would be gained by making cultures of the birds' bone-marrow.

VI. SUMMARY

1. A survey of 227 rooks (*Corvus frugilegus frugilegus* L.) and seventy jackdaws (*C. monedula spermologus* Vieill.) was conducted in Hertfordshire. The birds were examined by means of cultures of their peripheral blood, or (in the case of eleven rooks and one jackdaw only) bone-marrow, on N.N.N. medium. Twenty-seven rooks (11·9 %) and six jackdaws (8·6 %) were found to be infected.

Some of the rooks were examined at a time of year when the trypanosomes are not present in the peripheral circulation; other rooks were examined in the first

year of their life before adults of *Ornithomyia avicularia* (the vector of this parasite) were present. Ignoring birds of these two groups, twenty-six out of seventy-eight (33·3 %) adult rooks were found to be infected. This value probably approximates to the natural infection rate among adult rooks (and perhaps jackdaws).

2. The trypanosome in question is a large spindle-shaped form, measuring an average of 48·2 μ in length (excluding flagellum) and 5·5 μ in width. There is a tapering aflagellar region extending 14·1 μ (on the average) beyond the kinetoplast. This trypanosome is assigned to the species *T. avium* Danilewsky, 1885.

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(*MS. received for publication* 18. VI. 1955—Ed.)

STUDIES ON *TRYPANOSOMA AVIUM* DANILEWSKY 1885

II. TRANSMISSION BY *ORNITHOMYIA AVICULARIA* L.

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(With 1 Figure in the Text)

CONTENTS

		PAGE
I.	Introduction	321
II.	Previous work	322
III.	Materials and methods	322
IV.	Present work	325
	(i) Preliminary transmission experiments	325
	(ii) Confirmatory transmission experiments	326
	(iii) Experiments to demonstrate 'natural' transmission	327
	(iv) Experiments to test infectivity of mid-gut flagellates	327
	(v) Experiments to test transmission by the bite of the louse-fly	328
	(vi) Experiments to test whether birds can become infected orally	329
	(vii) Experiments to test infectivity of faecal matter	330
	(viii) Experiments involving arthropods other than *O. avicularia*	331
V.	Discussion	332
VI.	Summary	332
	References	333

I. INTRODUCTION

The first paper on *Trypanosoma avium* (Baker, 1956) reports the incidence of the trypanosome in some wild birds of Hertfordshire, while the present paper gives an account of the transmission of this flagellate by the hippoboscid 'louse-fly' *Ornithomyia avicularia* L. The third paper in the series contains a description of the life cycle of *T. avium* in both insect and vertebrate hosts.

My attention was drawn to this parasite during the course of some unpublished work on *Leucocytozoon*, when some individuals of *O. avicularia* were allowed to feed on a blackbird (*Turdus merula merula* L.) infected with *Leucocytozoon* sp. On subsequent dissection of two of these insects, a heavy infection of crithidial flagellates was found in their mid-guts. A fresh preparation of blood from the blackbird was then examined, and a trypanosome was seen. It was realized that if the flagellate infections of the insects were derived from that of the bird on which they had fed, the possibility existed that this species of *Ornithomyia* was a vector of the avian trypanosome. Work was therefore commenced to find out (*a*) if *O. avicularia* did in fact transmit the trypanosome, and (*b*) if so, by what route the transmission occurred. This work is described below.

II. PREVIOUS WORK

Danilewsky (1889), the first worker to describe trypanosomes from birds, suggested that young birds became infected from their parents orally, while the latter were feeding them. In 1904, Schaudinn published his account of the development of *T. noctuae* of the little owl in *Culex pipiens*. He described a very complicated life cycle, involving (according to Grassé, 1952, p. 637) spirochaetes, *Leucocytozoon*, and avian spermatozoa as well as trypanosomes. His work, although supported by two papers by Ed. & Ét. Sergent (1904, 1905), is now almost universally discredited. Novy, MacNeal & Torrey (1907) failed to infect birds in various ways with flagellates derived from naturally infected mosquitoes, and Woodcock (1910) considered the possibility of insects which were ectoparasitic on nestlings serving as vectors of some avian trypanosomes: one such insect which he mentioned was *Ornithomyia*. Later, however, Woodcock (1914) published an account of the development of *T. noctuae* in *Culex pipiens*, resulting in the formation of long thin trypanosomes which he considered were the infective forms, but he was unable to prove this by transmitting the infection to 'clean' owls, as his mosquitoes would not feed twice. Duke & Robertson (1912) found that *T. gallinarum* developed to crithidia in the mid-gut of *Glossina palpalis*. They considered that the normal vector of this trypanosome would prove to be a species of *Simulium*, and that *G. palpalis* was not, however, even a facultative vector. Nöller (1920) reported development of *T. loxiae* (of *Loxia curvirostra*) in culicine mosquitoes, but did not, apparently, attempt any transmission experiments with these insects. Aragão (1927) found crithidia in the guts of *Lynchia maura* (= *Pseudolynchia canariensis*) fed on a pigeon which was infected with *T. hannai*. He attempted to infect six pigeons by inoculations of emulsions of infected insects' intestines, or by the bite of infected insects, but failed. In 1929, Macfie & Thomson discovered that a trypanosome which they had found in canaries underwent development in *Dermanyssus gallinae* (Acarina), metacyclic trypanosomes appearing in the haemocoele of the mite. They apparently succeeded in transmitting this trypanosome to uninfected canaries by intraperitoneal inoculation of infected mites, but pointed out that their experiments were not conclusive owing to the uncertainty as to whether their 'uninfected' canaries were not, in fact, harbouring latent infections (all their canaries were examined by means of fresh blood preparations only). Manwell & Johnson (1931) published some results which 'tended to confirm' those of Macfie & Thomson. Finally, Herman (1945) suggested that *Stilbometopa impressa* (a hippoboscid) might transmit *T. avium* of quail (*Lophortyx* sp.), but offered no experimental proof.

I have already published a brief note on the transmission of *T. avium* by *O. avicularia* (Baker, 1955).

III. MATERIALS AND METHODS

A full description of the methods of trapping, maintaining and examining wild birds was given in the first paper of this series (Baker, 1956), and will not be repeated here.

The canaries (*Serinus canarius*) used in the present work were obtained from dealers, usually some time in advance of their use in the experiments. While in the laboratory, both before and during the experimental work, these canaries were kept in individual cages standing on small wooden blocks in a strong solution of the detergent 'Teepol'. This was done in order to prevent the passage of mites from one bird to another, in view of Macfie & Thomson's (1929) work on the role of mites as possible vectors of avian trypanosomes. At regular intervals, the canaries were transferred to clean cages which had been treated either by steam (under pressure) or by immersion in paraffin. At no time during the course of this work was an accidental mite infestation seen among these canaries.

During the period between their purchase and their use in experiments, all canaries were examined regularly by means of cultures of their peripheral blood, made on N.N.N. medium as described in the previous paper (Baker, 1956). The number of tubes inoculated with each bird's blood varied according to the length of time during which it was in the laboratory, but, when relevant, is stated among the experimental details given below. Frequently more than one tube was inoculated with any one sample of blood, hence this information is given in the paper as 'a total of so many culture tubes was inoculated, on so many different occasions'. Only those culture tubes which remained uncontaminated by bacteria or fungi are taken into account, unless flagellates developed in them also. Not one case of spontaneous trypanosomiasis was detected among all the canaries examined (over thirty). Since they were examined, on an average, fortnightly over periods of up to 6 months, it is extremely unlikely that any of the positive results obtained in the experiments described below could have been due to latent or accidentally transmitted infections.

The *O. avicularia* used in this work were obtained from wild birds (chiefly rooks and jackdaws), which had been trapped as described in the previous paper, in one of two ways. If the bird from which they were to be collected was required alive, it was anaesthetized with Nembutal (see Baker, 1956, for details of dosage), and then placed in a rectangular glass jar, measuring about 12 in. deep by 5½ by 5½ in. At the closed end of this jar, which was laid on its side, was a pad of cotton-wool soaked in ether, while the open end of the jar was covered by a flap of stout paper and the bird's head protruded through an X-shaped aperture cut in the centre of this flap. At intervals the bird was removed and the anaesthetized louse-flies, if any, were collected from the jar or shaken out of the bird's plumage. Provided they did not come into contact with liquid ether, the insects soon recovered. This method, referred to throughout this paper as the 'ether-treatment', is very similar to a method described by Williamson (1952) and quoted by Bequaert (1953, p. 7), but was devised independently by Mr R. Killick.

When many flies were required, the above method became tedious. Instead, large numbers of birds (sometimes 100 or more) were collected from the traps described in the previous paper, killed (usually manually), and placed in gauze-covered mosquito cages. As the birds' bodies cooled, the louse-flies which had been infesting them, left them, and were collected from the gauze by means of a conventional suction apparatus. If not used for experimental work immediately

after collection, the insects were kept in a gauze-covered cage in a dark, fairly cool, place. Under these conditions they survived for 2 or 3 days without food, and sometimes even longer.

Since the numbers of flies with natural infections varied, and were always less than 100%, insects which were to be used in transmission experiments were allowed to feed on infected birds for periods of from 1 to about 7 days. Each batch of flies to be infected was placed in a cardboard 'feeding box', which was then secured on to an area of skin (from which the feathers had been removed) on the back of an infected bird. The feeding boxes were modifications of those used by Mohamed (1952), and consisted of either circular ($1\frac{1}{2}$ in. diameter by $\frac{3}{4}$ in. deep) or rectangular ($2\frac{3}{4}$ in. by $1\frac{3}{4}$ in. by $1\frac{1}{4}$ in. deep) cardboard boxes: the smaller size was used chiefly on blackbirds, the larger on rooks and jackdaws. Several different types of box were made, but essentially all were constructed as follows (see Fig. 1). The open side of half of a box was covered with sandfly gauze (*G*) affixed with adhesive tape. In the opposite side of the box a small hole (*H*) was cut. To the two longer sides of the box, one or two rubber bands (*B*) were sewn, or otherwise fastened. A thin segment of a circle was cut out of the lower, free, edge of each of the two shorter sides of the box to enable it to fit more comfortably on to the back of a bird. After the flies had been inserted through the small hole (*H*), which was sealed down with gauze and adhesive tape, the box was placed on the bird's back so that the gauze surface was against the skin. The rubber band was passed transversely around the bird's thorax, behind the wings. Up to about forty flies were placed in the larger boxes, but often quite a large proportion (sometimes $\frac{1}{2}$ or $\frac{1}{3}$) failed to survive for 5 or more days. Flies were not used in transmission experiments until at least 5 days after their first infective feed; most were kept throughout this time on infected birds, but a few were maintained after infection on 'clean' birds. To assist in placing large groups of flies in the boxes, the insects were sometimes lightly anaesthetized with ether or chloroform vapour.

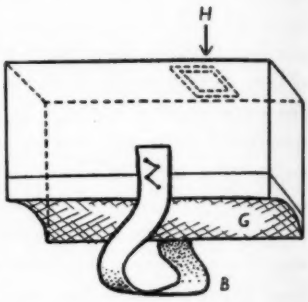

Fig. 1. Basic type of feeding box for insects. *B*, rubber band, passed around bird's body; *G*, sandfly gauze through which the insects fed; *H*, hole in top for introduction of insects.

After their removal from the feeding boxes, the surviving flies (sometimes after being kept at 30° C. for a short time) were lightly etherized or chloroformed, and dissected. When it was important to remove unbroken as much as possible of the alimentary canal, the method of dissection described by Mohamed (1952) for *Pseudolynchia canariensis* was used. When a large number of guts was required, and it did not matter if a few were broken, dissection was carried out by the much quicker method described by Hoare (1923) for *Melophagus ovinus*. For inoculation into birds, the required region of the gut was macerated with a ground glass pestle in a small amount of saline contained in a cavity pot, the resulting suspension being drawn up into a syringe and inoculated.

These descriptions of collecting, infecting, and dissecting louse-flies will not be repeated in the subsequent paper of this series.

IV. PRESENT WORK

(i) *Preliminary transmission experiments*

Following the initial discovery of flagellates in the mid-guts of two *O. avicularia* which had been kept for 7 days on a trypanosome-infected blackbird, as reported above (p. 321), attempts were made to produce trypanosome infections in canaries by inoculating them with suspensions of macerated guts of infected flies. The first of these attempts, which involved the inoculation of one macerated mid- and hind-gut of an infected fly intraperitoneally into an uninfected canary, failed. Subsequent examination of fresh blood preparations, and inoculations of blood into N.N.N. culture tubes, revealed no infection in the canary. It is now realized that with such a small inoculum a very weak infection might have developed which would have been overlooked in the examinations of blood preparations. As the culture tubes were not inoculated until 39 days after the bird's injection, such a weak infection (if it had developed) might have died out by that time.

This experiment was then repeated with more care. The mid- and hind-guts of eight *O. avicularia* collected (as before) from rooks and jackdaws in the aviary were dissected out and macerated in sterile Tyrode's solution. Microscopic examination of a drop of the suspension showed the presence of a large number of motile flagellates. Blood from a canary was inoculated into four culture tubes (three just prior to this experiment and one 4 days before), but flagellates did not develop in any of these tubes. The suspension (0·5 c.c.) was then injected intraperitoneally into this canary, and on the next day it appeared to be sick. Blood films were examined, both fresh and Giemsa-stained, and revealed the presence of many trypanosomes together with an infection of *Plasmodium hexamerium*. The latter, in view of the fact that this bird had at one time been used to maintain a strain of *P. hexamerium*, was assumed to be due to a relapse of an intercurrent infection. The canary was killed and autopsied, and stained impression smears of the spleen, kidney, lung, liver, heart muscle, bone-marrow (tibio-tarsal) and brain were made and examined. In all these preparations, with the exception of those of bone-marrow and brain, trypanosomes of the same type as those present in the peripheral blood were seen. In the smears of bone-marrow and brain, no flagellates were found. At autopsy, the spleen was seen to be very enlarged (18 × 7 mm.) and this, together with the symptoms exhibited by the canary, is now thought to have been caused by the relapsing malaria infection and not the trypanosomiasis.

Following this successful transmission experiment, an attempt was made to infect a young chick. An inoculum was prepared from the macerated mid- and hind-guts of nine *O. avicularia*, suspended in sterile Tyrode's solution. A drop of this suspension was examined microscopically, and many active flagellates were seen. A canary and a 3-day-old Rhode Island Red pullet each received 0·5 c.c.

of this inoculum intraperitoneally. The canary was shown to be previously uninfected by the fact that four N.N.N. culture tubes, inoculated with its blood 4 days earlier, remained negative. Both chick and canary were subsequently examined by means of fresh blood preparations and the canary was found to be infected on the second day after its inoculation. The chick remained uninfected, as was confirmed by the failure of flagellates to develop in culture tubes inoculated with its blood 6–14 days later.

At this stage, further work on transmission by *O. avicularia* had to be postponed, due to the seasonal disappearance of adult insects. Flies became available again in June 1954, but transmission experiments were not recommenced until the following month.

(ii) *Confirmatory transmission experiments*

An experiment was performed to show beyond all reasonable doubt that the flagellates in the gut of *O. avicularia* gave rise to the large trypanosomes found in the blood of birds. The two canaries used in this experiment had been in the laboratory for about 6 months, and during that time a total of thirteen culture tubes had been inoculated with the blood of one of these birds (G 6) on nine different occasions, and twelve tubes on eight occasions from the other bird (G 10): no flagellates developed in any of these cultures. The hind-guts alone of twelve *O. avicularia*, which had been experimentally infected from a rook, were removed and macerated in sterile 0·85% saline. Canary G 6 received an intraperitoneal inoculation of 0·4 c.c. of this suspension and, as a control, 0·4 c.c. of sterile saline was inoculated similarly into canary G 10. A fresh preparation of blood was made from each bird 6 hr. after inoculation: that from G 6 was negative, but that from G 10 was not examined. Further preparations were made 24 hr. after inoculation: trypanosomes were present in that from G 6, but not in that from G 10. One N.N.N. tube was inoculated with blood from G 10 on the fifth day, and another on the fifteenth day after the experiment: no flagellates developed in either of these tubes.

In the course of experiments designed to elucidate the development of this trypanosome in the bird, which will be reported in detail in a later paper, similar inocula, prepared from the hind-guts of infected *O. avicularia*, were injected intraperitoneally into canaries which had been shown previously to be uninfected by the examination of cultures of their blood. Of seventeen such experiments, a trypanosome infection in the recipient canary developed in fourteen cases. Of the three canaries in which no infection was detected, one died overnight following its inoculation, one was killed and autopsied only 6 hr. after inoculation, and one was examined by means of sections of certain organs only in which trypanosomes could not be recognized. Also, a 'first-year' rook developed an infection following the intracardiac inoculation of a suspension of macerated hind-guts of *O. avicularia*. All these results are discussed in detail in the next paper of this series.

It is felt that the evidence offered above is sufficient to prove the specific identity of the flagellates of the alimentary canal of *O. avicularia* with that of the

trypanosome of the peripheral blood of birds which is regarded as *T. avium*. In order to see if transmission would occur under conditions as near as possible to those prevailing in nature, the following two experiments were performed.

(iii) *Experiments to demonstrate 'natural' transmission*

A nestling rook had been captured in May 1954 (before any adult *O. avicularia* had reappeared after the winter) and reared by hand in the laboratory. Since its capture, a total of six culture tubes had been inoculated on four different occasions with the bird's blood but no flagellates developed in any of them. In July, this rook was placed in a screened cage into which were released nine experimentally-infected *O. avicularia*. Fresh preparations of blood from the rook were examined 1, 2, 4, 5 and 8 days later. On the eighth day, trypanosomes were seen in the blood. The bird was then removed from the screened cage, the inside of which was examined for the presence of louse-flies: none was found. The rook was then subjected to the 'ether treatment' for the collection of *O. avicularia* (see p. 323) and five were collected. It seemed likely that the four missing flies had been eaten by the rook.

A similar experiment was performed in October 1954. Three 'first-year' rooks (nos. 78, 79 and 80), which had been trapped during the previous September, and blood cultures from which were negative in three (in one case four) culture tubes, were placed in a screened cage into which four infected *O. avicularia* were released. Two days later, a fresh preparation of blood from each bird was examined and a trypanosome was seen in the blood of one of them (no. 78).

These experiments show that infected individuals of *O. avicularia* are capable of infecting 'clean' rooks under semi-natural conditions.

The experiments involving the inoculation of macerated hind-guts of infected flies indicated that the infective forms developed in that region of the insect's gut. This was confirmed by a study of the development of the parasite in the vector which will be reported in a later paper. However, two experiments were conducted to determine whether or not the numerous flagellates found in the mid-guts of infected flies were infective to birds.

(iv) *Experiments to test the infectivity of mid-gut flagellates*

The first of these experiments, involving the maceration of the mid-guts alone of infected flies and the inoculation of the resulting suspension intraperitoneally into an uninfected canary, was rendered inconclusive by the death of the canary (apparently from shock) 23 hr. after its inoculation. No flagellates were visible in the blood at the time of death, but a low-grade infection might have been overlooked. Furthermore, mid-gut flagellates might have been infective to canaries, but with a longer incubation period than that shown by the hind-gut forms.

Consequently, this experiment was repeated on a second canary (G 12) from which a total of fifteen negative cultures had been made on ten different occasions during the previous 6 months. This bird had previously had faecal matter from infected louse-flies rubbed into a scarified area on its skin (see below, p. 330), but no

infection had followed (eight fresh blood films and one culture tube being negative since this was done). The canary received an intraperitoneal inoculation of a suspension of macerated mid-guts from twenty-five experimentally infected *O. avicularia*. Fresh preparations of its blood were examined about 24 and 42 hr. later but no trypanosomes were seen. However, two N.N.N. culture tubes, inoculated with peripheral blood 24 hr. and 3 days respectively after the injection, became positive, indicating that a weak infection had developed. This might have been due to the presence of some hind-gut flagellates in the mid-gut by artificial regurgitation during dissection. It is realized that the previous history of the canary might have had some bearing on the course of a subsequent infection, although this is not thought likely.

Infection having been shown to occur following the intraperitoneal injection of flagellates found in the hind-guts of louse-flies which had fed on blood infected with trypanosomes, it remained to discover the route by which birds become contaminated. The apparent localization of the infective forms in the hind-gut of the insect suggested that infection might occur by one of the following routes: (*a*) faecal contamination of the puncture caused by the bite of the fly or other wounds in the bird's epidermis; (*b*) orally, following the ingestion of the insect by the infested bird, as occurs in the case of *T. melophagium* of the sheep (Hoare, 1923). It was realized, however, that the possibility of infection occurring via the insect's proboscis during feeding could not be ruled out. The following experiments were performed in an attempt to find out by which of these routes infection did occur.

(v) *Experiments to test transmission by the bite of the louse-fly*

The rook used in this experiment had already had two groups of *O. avicularia* fed upon it, some of the second group being infected with flagellates. Between the feeding of these two groups of flies, the rook had been kept in an insect-proof aviary and a total of twenty-one N.N.N. tubes had been inoculated with its blood on eleven different occasions; no flagellates developed in any of these tubes. After the second group of flies had fed upon the rook, another culture tube was inoculated with its blood and remained negative. Thirty *O. avicularia* (fed upon an infected rook) were then placed in a feeding box attached to the experimental rook. Five days later, twenty-three flies were still alive; of these, eleven were removed for use in other transmission experiments. The twelve remaining flies were left on the rook for a further 6 days (making 11 days in all). The box was then removed and three flies were found to be alive. Four had apparently escaped through a small hole in the gauze of the box. Accordingly, the bird was searched for flies but only one (an infected female) was recovered. A fresh preparation of the rook's blood was examined, with negative results, 4 days after the beginning of the experiment and seven culture tubes were inoculated with its blood between 5 and 33 days after the experiment began; no flagellates developed in any of these tubes. This rook was later infected with trypanosomes by means of an intracardiac inoculation of macerated louse-fly hind-guts, thus showing that its failure to become infected in the above experiment was not due to an immunity.

This result was confirmed by some observations made during the course of the experiments designed to elucidate the developmental cycle which the trypanosome undergoes in the insect. Four *O. avicularia* were allowed to feed on an infected rook, and were then placed in a feeding box on an uninfected juvenile rook. One of these four flies was removed about 24 hr. later, one after about 48 hr., one after 4 days, and one after 7 days (on the accidental death of the rook). All these four flies were infected. An N.N.N. culture tube, inoculated 1 day after the flies were first placed on the bird, remained negative. At the death of the bird, culture tubes were inoculated with heart blood and bone-marrow, but no flagellates developed in either, thus showing that the bird had not become infected.

(vi) *Experiments to test whether birds can become infected orally*

The hind-guts of nineteen *O. avicularia* (which had been kept on infected birds) were removed and macerated in 0·2 c.c. of sterile normal saline. 0·15 c.c. of this suspension was then introduced, from a syringe fitted with about 3 cm. of narrow polythene tubing, into the mouth and oesophagus of a canary (G 9). During the 6 months since its purchase, a total of thirteen culture tubes had been inoculated with blood from this canary, on nine different occasions, the last being the day of the experiment. No flagellates had developed in any of these tubes. As a control, 0·15 c.c. of sterile normal saline was introduced similarly into another uninfected canary (G 15) the next day. The experimental canary (G 9) was examined by means of fresh preparations of peripheral blood at 4, 18¾, 20, 21, 22¼, 23, 24, 25 and about 43 hr. after inoculation. Trypanosomes were seen in the preparations made at 22¼, 24, 25 and 43 hr. In the first three of these positive preparations, the trypanosome seen was similar in shape to the normal blood form, but smaller. In the preparation made the following morning (43 hr.) a normal, large trypanosome was seen. The control canary (G 15) remained uninfected, as was shown by the fact that no flagellates developed in culture tubes inoculated with its blood 1, 4 and 8 days after its injection with saline. This demonstrates that infection follows the oral introduction of infective material into birds.

As a corollary to this experiment, one was performed to see if infection would result from a bird's feeding on entire infective flies. The only bird available, which was both uninfected with trypanosomes and insectivorous, was a mistle-thrush. It had been captured as a nestling in May 1953 and kept since that time in an insect-proof aviary. Unfortunately this bird had previously received two inoculations, one of blood from a blackbird infected with trypanosomes, and one of macerated organs from a rook which was not infected with trypanosomes. The blood injection was given in August 1953, and did not give rise to an infection. After this inoculation, the bird had been examined by means of fresh blood preparations, 1, 3 and 11 days later and N.N.N. culture tubes 1, 1½, 5½, 6, 6½, 7 and 9 months later. All these examinations were negative. In June 1954 the tissue suspension was inoculated (for purposes not connected with the present work). Blood cultures were made from the mistle-thrush 1 and 4 weeks after this: no flagellates developed in these cultures. Thus the bird was considered to be uninfected.

For the present experiment, performed in July 1954, thirty-six *O. avicularia* which had been kept on infected birds were very lightly chloroformed. Their wings were removed, and the flies were placed in a dish which was stood in a larger dish containing water, to prevent their crawling away. The legs of the flies were not removed since a previous attempt had shown that the bird would not eat non-motile, legless insects. The dish of insects was then placed in a cage with the mistle-thrush which had been offered no food since the previous day. Thirty-three minutes later all but one (dead) fly had disappeared. It was assumed that the bird had eaten the rest. Fresh preparations of blood from the thrush were then examined at approximately hourly intervals from about 18 to 24 hr. after the bird had eaten the flies; no trypanosomes were seen in any of these preparations. A similar preparation was examined about 40 hr. after the bird had ingested the insects and a trypanosome was seen. In my opinion, this infection was almost certainly due to the eating of the louse-flies, since it is most unlikely that an infection would have remained latent for 11 months following the bird's inoculation with infected blood without being detected in some of the many culture tubes inoculated during that time.

(vii) *Experiments to test infectivity of faecal matter*

On occasions, flagellates have been seen in faecal matter extruded from the anus of infected flies by gentle pressure on the abdomen. Also, flies have been observed to defaecate shortly after, although not while, feeding. It was decided therefore to see if infection would follow the contamination of wounds in the skin of birds with faeces from infected flies.

Faeces were collected by gentle ventral abdominal pressure from flies which had been kept on infected birds in the usual way. Thirty-nine insects were used, faeces being obtained successfully from twenty-five. The flies were afterwards used in the two experiments described immediately above, which afforded a check of their infectivity. The faecal matter obtained was emulsified in a little sterile normal saline and rubbed on to an area of skin on the breast of a canary (G 12); the feathers had previously been removed from this area, and the skin had then been lightly scarified with a needle. After a few minutes, surplus moisture was wiped gently off the area, to minimize the danger of oral contamination of the bird during its preening operations. Microscopic examination of both fresh and stained preparations of the faecal suspension revealed no flagellates. This canary (G 12) had been in the laboratory for 6 months, during which time a total of fourteen culture tubes had been inoculated with its blood on nine different occasions. No flagellates developed in any of these tubes. Between 16½ and 24 hr. after the experimental inoculation, eight fresh blood preparations from this bird were examined and no trypanosomes were seen. Five days later, one N.N.N. tube was inoculated with the canary's blood and remained negative. Thus, no infection developed in this canary. As a control, a second canary had sterile normal saline rubbed into a scarified area of the skin of its breast, and was subsequently examined by means of three culture tubes, all of which remained negative.

This experiment was twice repeated, without the use of a control canary. For the first repetition, faeces were collected from ten *O. avicularia* which had been kept on infected birds. Microscopic examination of a fresh drop of the faecal suspension did not reveal any flagellates. The rest of this suspension was then rubbed lightly into a scarified area of the skin on the breast of canary G 14. This canary had been in the laboratory for 4 months, during which time two negative cultures of its blood had been made, on two different occasions. The bird was examined by means of fresh preparations of blood at 1 and $2\frac{1}{2}$ days after inoculation and no trypanosomes were seen. One week after inoculation, an N.N.N. culture tube was inoculated with the canary's blood and remained negative.

A second attempt was made to infect this canary (G 14) in the same way, 9 days after the first. Faecal matter was collected, as before, from fourteen infected flies, emulsified in saline and rubbed into a scarified area of skin on the bird's breast (on the opposite side to that which was used previously). A drop of this inoculum, when examined microscopically, was seen to contain a few flagellates. The canary was examined by means of fresh blood preparations 1 and 2 days after the inoculation and no trypanosomes were seen. Unfortunately, all culture tubes inoculated with this canary's blood just after this experiment became contaminated with bacteria. It is therefore impossible to state that a weak infection did not develop in the canary. This is particularly regrettable as the faecal inoculum used in this case was the only one in which the presence of flagellates was demonstrated microscopically. However, these experiments indicate that this method of transmission must, at the most, play only a minor role in the spread of this infection.

(viii) *Experiments involving arthropods other than* Ornithomyia avicularia

No systematic investigation of the possible role of other arthropods in the transmission of *T. avium* was undertaken, but the following observations may be of interest.

Macfie & Thomson (1929) apparently succeeded in transmitting a trypanosome, which morphologically resembled that with which I worked, to canaries by means of the mite *Dermanyssus gallinae*. A species of blood-sucking mite was found on one rook and one jackdaw kept in an aviary during the course of the work described in this paper. This mite was kindly identified by Dr G. Owen Evans of the British Museum (Natural History) as *Ornithonyssus sylviarum*. About twelve of these mites, collected from the jackdaw (which was infected with *T. avium*) and about six of the mites collected from the rook (which was not infected with trypanosomes) were crushed in saline and the resulting suspension was examined microscopically. No flagellates were seen in either of these preparations.

A few experiments were conducted with some nymphs of *Rhodnius prolixus* (Reduviidae) to see if transmission by this insect could be used as a means of obtaining heavy infections in canaries when adult *O. avicularia* were not available. Some nymphs were allowed to feed on a trypanosome-infected rook and then maintained in a moist atmosphere at 30° C. Two nymphs were dissected at 20 and 33 days, respectively, after feeding, and their gut contents were examined in

saline. No flagellates were seen. Twenty-one days later, two of these nymphs were fed again on a fairly heavily infected canary and dissected 12 and 21 days later, respectively. Again, no flagellates were found in their mid- or hind-guts. No further experiments were performed, it being concluded that this trypanosome would not develop readily in *Rhodnius prolixus*.

Attempts were made to get some *Culex molestus* and *Aedes aegypti* to feed on an experimentally infected canary. Only one of the *Culex molestus*, which were placed in a cage with the canary for 12 hr., did feed. This insect was examined 48 hr. after its removal from the canary cage, but no flagellates were found in its mid- or hind-gut.

V. DISCUSSION

There seems to be little doubt, from the work reported above, that *Ornithomyia avicularia* is a vector of avian trypanosomes in this country. *O. avicularia* is a member of the family Hippoboscidae ('louse-flies'), and is described and figured by Edwards, Oldroyd & Smart (1939, p. 120 and pl. 40). Much information concerning it can be obtained from Bequaert (1953). Other members of this family have been reported as vectors of trypanosomes—*Melophagus ovinus* for *Trypanosoma melophagium* of sheep (Hoare, 1923), and *Lipoptena caprina* for *T. theodori* of goats (Theodor, 1928).

As avian trypanosomes have been reported frequently from America, and *O. avicularia* is restricted to the Old World, it seems highly probable that other species of hippoboscids serve as vectors: the work of Aragão (1927) suggests that *Pseudolynchia canariensis* may be one of these. *Stilbometopa impressa* may well be another (see Herman, 1945). In view of this, it is interesting to note that Bequaert (1953, p. 15) regards the tsetse flies (Glossinidae) as possibly the closest living relatives of the Hippoboscidae. It might prove interesting to re-examine the possibility of cyclical development of *T. theileri* occurring in *Hippobosca* spp., originally suggested as vectors of this cattle trypanosome by Theiler (1903).

The work of Macfie & Thomson (1929) suggests that other arthropods may also serve as vectors of some avian trypanosomes. Further work on the matter of host-specificity in such trypanosomes during both stages of their life cycle would be of interest. Further work is also needed on the route of infection of birds. Although there seems little doubt, from the results presented above (pp. 329–30), that ingestion of infective louse-flies is the chief means whereby birds become infected, the non-infectivity of faecal matter from infected flies needs confirming. Also, it would be interesting to see if infection would follow the oral introduction of infected insects' faeces into birds.

VI. SUMMARY

1. It is shown that *Ornithomyia avicularia* L. (Diptera, Hippoboscidae) is a vector of *Trypanosoma avium* in this country.

2. The metacyclic trypanosomes develop in the insect's hind-gut, and infect birds by penetrating the membranes of buccal cavity and/or oesophagus and crop.

3. The opportunity for infection to take place occurs when birds ingest infected insects.

4. The significance of this finding in connexion with other reports of (*a*) vectors of avian trypanosomes, and (*b*) trypanosomes transmitted by hippoboscids, is discussed.

5. Attempts to obtain development of *T. avium* in a few nymphs of *Rhodnius prolixus* and one adult *Culex molestus*, failed. Mites from an infected bird were examined for the presence of flagellates, with negative results.

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* Not seen in the original.

(*MS. received for publication* 18. VI. 1955.—Ed.)

STUDIES ON *TRYPANOSOMA AVIUM* DANILEWSKY 1885

III. LIFE CYCLE IN VERTEBRATE AND INVERTEBRATE HOSTS

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(With 5 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	335
II.	Previous work	335
III.	Materials and methods	336
IV.	Present work	338
	(*a*) Development in the bird	338
	(i) Course of infection	338
	(ii) Habitat of the trypanosomes when absent from the peripheral circulation	339
	(iii) Do the trypanosomes multiply in the bird?	340
	(iv) Habitat of the trypanosomes during the 'pre-patent' period	343
	(*b*) Development in *Ornithomyia avicularia*	346
	(i) Development in the mid-gut	346
	(ii) Development in the hind-gut	349
V.	Discussion	350
VI.	Summary	351
	References	351

I. INTRODUCTION

This is the third paper of a series describing an investigation of *Trypanosoma avium* in English birds. The two previous papers (Baker, 1956*a*, *b*) dealt with the description and identification of this trypanosome, the results of a survey of its incidence among corvid birds of Hertfordshire, and its transmission by *Ornithomyia avicularia*, in which it undergoes a cyclical development. The present paper describes this development, together with that undergone by *T. avium* in its avian host.

II. PREVIOUS WORK

Several authors have described, in more or less detail, developmental cycles undergone by trypanosomes in birds. Danilewsky (1889) observed in fresh preparations of blood, etc., what he regarded as such a development, but this appears to have been actually the early stages of the development which occurs in the vector. Schaudinn (1904) published a confused account of the development of *T. noctuae* in the little owl, much of which is now considered erroneous. Thiroux (1905) published a full account of *T. paddae*, in which the only development recorded from the avian host was binary fission in the peripheral blood, found in only very heavy infections.

Woodcock (1910) described a polymorphic trypanosome (*T. fringillinarum*) from finches, in which the various forms seen were linked up into a rather complex

hypothetical developmental cycle. The only signs of multiplication which Woodcock actually saw were a few trypanosomes possessing a double kinetoplast. A similar cycle was described by Minchin & Woodcock (1912) for *T. noctuae*. (These authors were, incidentally, unable to confirm Schaudinn's work.) Here, again, although the only signs of multiplication seen were a very few forms with double kinetoplasts (including one with an apparently double karyosome), hypothetical processes of multiple as well as binary fission were invoked in the developmental cycle. The majority of authors have recorded their failure to find forms undergoing division in infected birds.

Accounts of development in the vector are decidedly few. Schaudinn (1904), supported by the Sergents (1904, 1905) and others, described the development of *T. noctuae* from ookinetes of *Haemoproteus* and *Leucocytozoon*, and their subsequent metamorphosis into spirochaetes, in *Culex pipiens*. Later work has discredited this. Woodcock (1914) described the development of *T. noctuae* in *C. pipiens* in more conventional terms, involving a multiplicative phase as crithidia and the subsequent production (after 34 hr.) of long narrow trypanosomes, which he regarded as the phase infective to other owls. He was, however, unable to confirm this by transmitting the parasite to other owls as his mosquitoes would not feed twice. Nöller (1920) reported a serial development of *T. loxiae* in *Culex*, resulting in the appearance of attached flagellates in the hind-gut. Macfie & Thomson (1929) described the development of a trypanosome from canaries in the mite *Dermanyssus gallinae*. The cycle undergone by the flagellate resulted in the formation of metacyclic trypanosomes after 3 or 4 days in the body cavity of the mite, the gut at this time being devoid of flagellates. The developmental stages of this trypanosome appear to resemble quite closely those described below (pp. 346–50) for *T. avium*.

III. MATERIALS AND METHODS

Methods of trapping, examining and maintaining wild birds have already been described in the first paper of this series (Baker, 1956*a*); methods of collecting, infecting and dissecting *O. avicularia*, and housing and examining canaries, were reported in the second paper of this series (Baker, 1956*b*).

In the work reported in this paper, fresh preparations of small pieces of organs from infected birds were made by maceration in saline on a slide in the usual way. Dried impression smears were also made, fixed with methanol and stained with Giemsa's stain. Some birds were autopsied with sterile instruments and, taking sterile precautions as far as possible, pieces of their viscera were inoculated into tubes of N.N.N. medium (prepared as described in Baker, 1956*a*). It was found to be almost impossible to avoid contamination of the tubes with bacteria, however, although in some cases flagellates developed together with the bacteria.

Counts were made of trypanosomes in the blood of infected birds, and of metacyclic trypanosomes in inocula prepared from the macerated hind-guts of infected *O. avicularia*, using a modification of the normal method of enumerating leucocytes. Trypanosomes in blood were counted by diluting the infected blood one part in twenty with citrated saline solution (1·5% sodium citrate in 0·85%

saline) in a leucocyte-counting pipette. A count of the trypanosomes present in this diluted blood was then made on a Neubauer haematocytometer slide. It was found advisable to count over the entire ruled area (9 mm.^{2}) on the slide—i.e. to examine 0·9 mm.^{3} of diluted blood. Four such counts were made, and their results averaged. This average was then multiplied by 200 and divided by nine to obtain the number of trypanosomes per cubic millimetre of undiluted blood. As the trypanosomes were alive during the counting, care was taken to alter the focus of the microscope continually to include all the trypanosomes present.

In order to test the accuracy of this method, on three separate occasions pairs of pipettes of diluted blood were prepared from the same bird almost simultaneously, and counted consecutively. The three pairs of results so obtained (expressed in trypanosomes per cubic millimetre of undiluted blood) were: (i) 110 and 90; (ii) 915 and 810; (iii) 790 and 715. The fact that, in all three of the above trials, the lower value was obtained from the second reading, suggests that the delay of about 1 hr. while the first tube was being counted may have contributed somewhat to the discrepancy; if so, the accuracy of this method could be assumed to be greater than the above examples would suggest. Although it is clear that great accuracy cannot be expected from this method, it was the best available for counting trypanosomes present in such (relatively) small numbers.

A similar method was used to count metacyclic trypanosomes in inocula. First, a count was made of all flagellates present in the inoculum. This was done by diluting a drop of the latter in a leucocyte-counting pipette, either one part in ten or one in twenty, with 0·85 % saline containing a trace of 2 % osmic 'acid' (OsO_4) solution (which killed the flagellates). The count was performed as for a leucocyte count, using the four ruled areas of 1 mm.^{2} each on a Neubauer slide. The four results were averaged and converted by suitable multiplication to the total number of flagellates inoculated into the bird. The percentage of these forms which were metacyclic trypanosomes was then obtained by examination of a dried, stained film of the inoculum. Thus the approximate number of metacyclic trypanosomes introduced into the bird could be calculated.

Smear preparations were made from various regions of the gut of insects by thorough teasing in saline on a slide, and spreading the suspension. Sometimes these preparations were dried rapidly (by waving) and treated as dried blood films. Better results were obtained, however, when they were treated by a modification of the method given by Minchin (1909), as follows. The still-wet preparations were fixed by immersion in the vapour of a 2 % solution of osmic 'acid' in a closed jar for about 20 sec., and were then immersed in absolute ethanol for 15 min. After hydration in graded alcohols, the films were stained for about 24 hr. in a dilute solution of Giemsa's stain (one drop of stain to 1 c.c. of distilled water, pH 7·2). After rinsing in water, the films were differentiated with Orange G solution: the optimum time for differentiation was usually about 10 sec. After washing in water and dehydrating in an acetone-xylol series, the films were cleared in xylol, rinsed in Euparal essence, and mounted in green Euparal. This method is referred to in this paper as the 'wet-fixed' process.

Sections were cut of both entire *O. avicularia* and their dissected alimentary canals. For sectioning entire, flies (with wings and legs removed) were fixed in Carnoy's fluid for 1 hr., and then double-embedded in celloidin and paraffin wax

according to the conventional technique. Isolated guts of infected flies were handled as suggested by Hoare (1923), being usually fixed in Carnoy's fluid for 5 or 10 min. For staining sections of entire flies or isolated guts, the Giemsa-colophonium method of Shortt & Cooper (1948) was found less satisfactory than haematoxylin and eosin staining, since the Giemsa's stain was so heavily absorbed by the insect tissues as to make differentiation very difficult to perform without completely decolorizing the flagellates.

IV. PRESENT WORK

(*a*) *Development in the bird*

(i) *Course of infection*

All experimental infections in canaries followed similar courses in general. After a prepatent period varying from 17 to 24 hr., flagellates would appear in the blood stream. These trypanosomes were of the same general shape as those seen in a mature infection, and described in the first paper of this series (Baker, 1956*a*), but were smaller. The parasites were apparently almost fully grown by about 2 days after inoculation. Even these experimental infections never became intense, the highest recorded parasitaemia being in a bird with 865 parasites per mm.^{3} of blood (see graphs, Fig. 1). After this peak had been reached parasitaemia declined gradually, sometimes with some fluctuations, until, generally between 1 and 2 months after the start of the infection, parasites could no longer be found in the peripheral blood. Two canaries were examined by means of blood cultures, one at about 4, 5, 6 and 8 weeks, and the other at about 8, 9, 10 and 12 weeks after the start of the infection: no flagellates developed in any of these cultures. The first of these birds was killed at 8 weeks and a culture and microscopic preparation of its bone-marrow were made and examined. No flagellates were found in either, which suggested that the disappearance of the parasites from the blood stream was absolute. This is interesting in connexion with the observations upon naturally infected Corvidae reported below. The 'control' curve in Fig. 1 A shows graphically a typical experimental infection in a canary.

The infections occurring naturally in rooks and jackdaws were difficult to study because the trypanosomes were always very scanty. Frequently birds would be shown to be infected by means of cultures of their blood, whereas microscopic examination of fresh blood preparations failed to reveal any trypanosomes. Observations upon a jackdaw and a blackbird suggested that natural infections were not self-limiting, as the experimental infections in canaries seemed to be. The blackbird, which was found to be infected while held in captivity, gave positive blood cultures in September, December (1953) and January (1954). During February 1954 cultures inoculated with its blood remained negative, but in April (none were made in March) and May they were again positive. These observations were continued during the next autumn and winter. In October and December 1954, only negative cultures were obtained. In January of 1955, however, flagellates developed in a culture tube, but tubes prepared in February and early March remained negative. Tubes inoculated at the end of March and during April (1955) became positive.

A similar course was followed by the infection of a jackdaw whose blood gave rise to positive cultures during October and November 1954. Tubes inoculated in December remained negative, but two tubes inoculated in January and February (1955) became positive. A further two tubes, inoculated on 18 February and 9 March, remained negative, but tubes inoculated on 31 March and 14 April (1955) became positive. On 14 April fresh blood preparations from both the blackbird and the jackdaw were examined, but no trypanosomes were seen. Neither of these birds was kept in a screened aviary during the time that these observations were made, but it seems most unlikely that this reappearance of parasites in their blood streams could have been due to reinfection, bearing in mind the time of year at which it occurred. The occurrence of a temporary relapse during January 1955 in both cases is noteworthy. Observations on both birds are continuing at the time of writing.

(ii) *Habitat of the trypanosomes when absent from the peripheral circulation*

If it is true that the trypanosomes leave the peripheral circulation of naturally infected birds during the winter months, only to reappear in spring or early summer, the problem arises as to their whereabouts during this period.

The first clue to an answer to this question was given by the examination of a rook which died on 2 April 1954. Two months earlier it had received injections of cultivated forms of *T. avium* and cortisone acetate. A culture tube inoculated with its blood 22 days after the cultures injection was positive, but another, inoculated 5 days before the bird's death, remained negative. It is uncertain whether this was a transient infection due to the injection of culture fluid, or a temporary reactivation of a latent infection by the cortisone. The bird died 5 days after the last culture tube was inoculated with its blood and at autopsy a normal 'blood-type' trypanosome was seen in its bone-marrow. Examination of dried, stained impression smears of all viscera and bone-marrow failed to reveal any trypanosomes. One other rook which had shown trypanosomes in a blood film in September 1953, but had negative cultures prepared from its blood in December of that year and in January and February of 1954, died in the February. Dried and stained impression smears of the viscera of this bird (including bone-marrow) failed to reveal any trypanosomes.

The following autumn, an experiment was carried out on a jackdaw from which positive cultures had been made at intervals between 26 April (when it was trapped) and 13 October 1954. Cultures made on 11 and 17 November remained negative. On 25 November 1954, the bird was killed. At autopsy, pieces of various organs were inoculated (with as many sterile precautions as possible) into tubes of N.N.N. medium: fresh preparations of various organs (macerated in saline), as well as dried and stained impression smears, were made. Trypanosomes were seen by microscopical examination in the fresh preparation of bone-marrow only. The only culture tubes which remained uncontaminated by bacteria were those inoculated with pieces of liver, spleen and bone-marrow. Flagellates developed in none of these. (Their absence from the bone-marrow tube suggests a failure of the technique used.) However, flagellates developed in a

tube inoculated with a piece of lung, although this tube was contaminated with bacteria.

Such evidence as is available, therefore, suggests that the parasites, at least in some cases, pass the winter months more-or-less restricted to the bone-marrow and possibly also other organs of the host. On occasions, however, they may be found in the peripheral blood (see pp. 338–9).

(iii) *Do the trypanosomes multiply in the bird?*

In all the infections which have been examined, both natural and artificial, no trypanosomes undergoing division have been seen. This, coupled with the fact that intense infections have never been noted, even in experimentally infected canaries given relatively large inocula, led me to question whether or not the trypanosome did multiply in its avian host. The following work was done in an attempt to answer this question.

It was felt that the failure to observe dividing trypanosomes might be due to (*a*) the fact that multiplication occurred elsewhere than in the peripheral blood, (*b*) the fact that it occurred there, but only rarely, or (*c*) the fact that it did not occur at all. If the first suggestion were true, the failure of intense infections to appear might be due to (i) active lysis and/or phagocytosis by the host, or (ii) division occurring only sporadically. An experiment was performed which, it was hoped, would point to one or other of these possibilities.

Three previously uninfected canaries were given, intraperitoneally, an inoculum prepared from the hind-guts of twenty-six *O. avicularia*. A count carried out on this inoculum (by the method described above, p. 336) showed that each bird received about 800,000 metacyclic trypanosomes. One of these birds (G 17) received intraperitoneal inoculations of 0·5 c.c. of an approximately 0·25% suspension of 'Chinese ink' (finely divided carbon—see Rao, 1951) 2½ days and 5¼ hr. before the flagellate inoculation. By mechanically 'blocking' the reticulo-endothelial cells of the bird with carbon particles, it was hoped that the effects of phagocytosis (if any) on the subsequent parasitaemia would be lessened. The second canary (G 15) received two intraperitoneal injections of 0·5 c.c. of a 20% solution of glucose in normal saline, one at 2 hr. before the flagellate inoculation and one 20 hr. after it. It has been shown by Poindexter (1933) that glucose injections increase the parasitaemia in *T. equiperdum* infections of guinea-pigs, presumably through the increase in available blood-sugar. It was hoped that the administration of glucose to this canary would increase the rate of reproduction of the avian trypanosome, if any was occurring. The third canary (M 22A) received no special treatment and served as a control.

Commencing on the second day after the inoculation of the trypanosomes, counts were carried out of the numbers of flagellates in the peripheral blood of these three birds. These results are expressed graphically in Fig. 1A, from which it can be seen that no significant rise in parasitaemia was recorded in either of the experimental birds (G 15 and G 17): in fact, both had slightly lower parasitaemias than the control bird, although no significance is attached to this. The graph also shows that in none of these birds did the number of parasites per c.c.

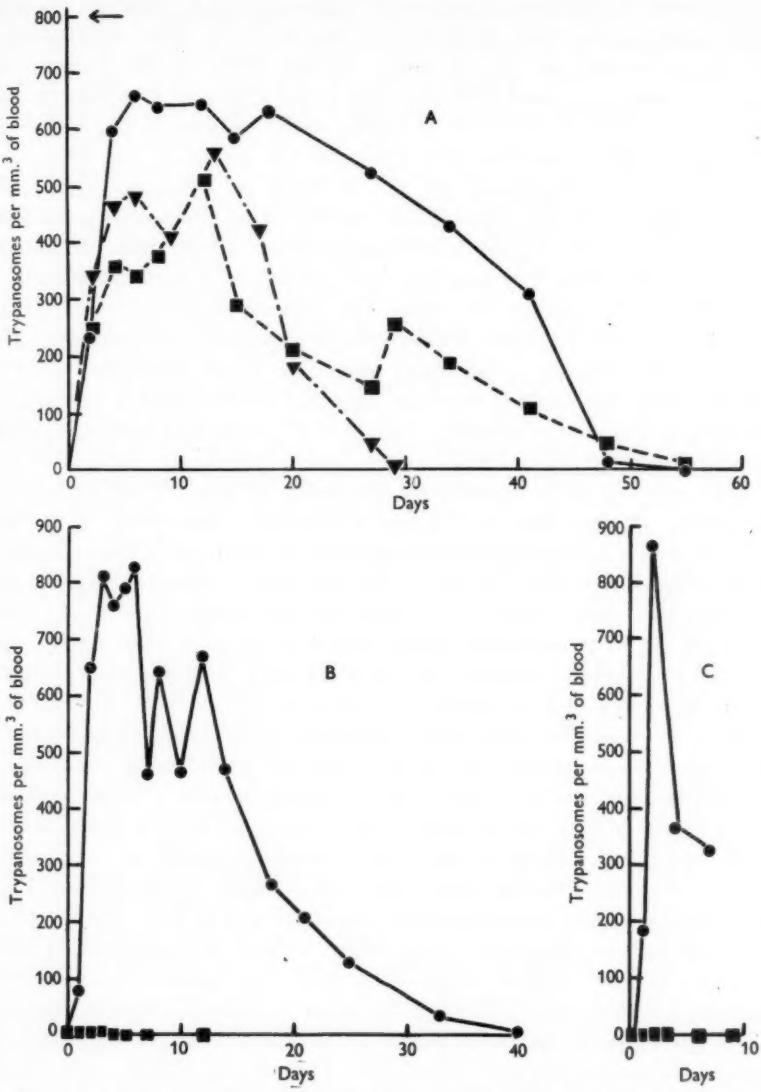

Fig. 1. Graphs showing parasitaemia in experimental infections with *T. avium* in canaries. Intraperitoneal inoculations of metacyclic trypanosomes given on day 0 in each case. A. Arrow indicates approximate number of metacyclic trypanosomes inoculated to each canary (800,000), expressed per mm.³ of recipient's blood. ●——●, control canary (M 22 A); ▼—·▼, canary receiving injections of carbon ('Chinese ink') (G 17); ■— —■, canary receiving injections of glucose (G 15). B. Inoculum of about 1,500,000 metacyclic trypanosomes given to each canary. Day 0 = day 29 of graph A. ●——●, control (previously uninfected) canary (G 21); ■——■, canary with previous infection (G 17). C. Inoculum of about 2,650,000 metacyclic trypanosomes given to each canary. Day 0 = day 61 of graph A. ●——●, control (previously uninfected) canary (G 30); ■——■, two previously infected canaries (G 15 and M 22 A).

of blood exceed the number of metacyclic trypanosomes inoculated. The highest recorded parasitaemia, that of M 22A on the sixth day after inoculation, was 660,000 trypanosomes per c.c. of blood. Hewitt (1940, p. 39) gives the blood content of a canary as about 1000 mg. This would represent a volume of slightly less than 1 c.c. Since no evidence of higher concentrations in any tissues other than the peripheral blood has been found at the height of the infection, the estimated number of flagellates per c.c. of blood (at maximum parasitaemia) is taken, tentatively, as the approximate number of trypanosomes present in the bird's body at that time. Thus it would appear that in all the three canaries of the above experiment, fewer trypanosomes were present than were inoculated.

In an attempt to confirm this work, two further experiments involving the inoculation of counted numbers of metacyclic trypanosomes into canaries, followed by counts of the numbers of flagellates appearing in the peripheral blood, were performed. These experiments were also designed to determine if any immunity to superinfection existed in canaries. Canary G 17, which had been infected in the experiment described immediately above, and a previously uninfected canary (G 21) both received an intraperitoneal inoculation of about 1,500,000 metacyclic trypanosomes, 29 days after G 17's first inoculation. The results of the counts made subsequently of the numbers of flagellates in the blood of these canaries are recorded graphically in Fig. 1B. It can be seen that a relatively heavy infection developed in the 'clean' canary, G 21 (max. 830,000 parasites per c.c. of blood on day 6), while G 17's parasitaemia, which was at a low level (6000 per c.c.) at the start of the experiment, persisted at this level for 3 days and then dropped to zero. No flagellates had reappeared in its blood by 12 days after its second inoculation. It should be noted that the highest parasitaemia recorded for G 21 was well below the estimated number of metacyclic trypanosomes inoculated.

The other two canaries which had been infected in the first of these experiments, namely G 15 and M 22A, and a 'clean' bird (G 30) were used in the final experiment of this group. Each of the three canaries received an intraperitoneal inoculation of about 2,650,000 metacyclic trypanosomes (from thirteen *O. avicularia*) 61 days after the first inoculation into G 15 and M 22A. The latter bird's infection had become subpatent 1 week earlier, while G 15's parasitaemia had only just declined to zero. Neither of these two previously infected birds showed any trypanosomes in their blood following this second inoculation, whereas the control bird (G 30) developed a maximum parasitaemia of about 865,000 parasites per c.c. of blood on the second day after inoculation. These results are shown in the graph of Fig. 1C. The number of parasites declined between the second and fourth days (as shown by the graph) more rapidly than is usual. Five blood counts had been performed on the canary during this period, and consequently it had become markedly anaemic: the sudden fall in the number of parasites was presumably due to their mechanical removal in the blood. The fact that their numbers had not increased again during the next 3 days appears to offer additional evidence that the trypanosome does not multiply in canaries.

(iv) *Habitat of the trypanosomes during the 'pre-patent' period*

Reference has already been made to the existence of a pre-patent period of between 17 and 24 hr., between the intraperitoneal inoculation of metacyclic trypanosomes and the appearance of flagellates in the peripheral blood. A similar pre-patent period existed in the case of a canary inoculated intra-oesophageally, as reported in the previous paper of this series (Baker, 1956*b*). It was realized that observation of the flagellates during this period was essential before definite conclusions about the occurrence or otherwise of multiplication in the avian host could be made. On the assumption that this period represented the time taken by the flagellates in migrating from the peritoneal cavity to the vascular system, a previously uninfected rook was given an intracardiac injection of about 6,000,000 metacyclic trypanosomes (from 50 *O. avicularia*). It was intended to follow the growth and development of the flagellates in preparations of the blood of this rook made at intervals during the first 24 hr. of its infection. Fresh blood preparations were examined immediately after the inoculation and at $\frac{1}{2}$, 3 and 6 hr. later without any flagellates being seen. However, one trypanosome was seen in a preparation made 22 hr. after the inoculation was given to the bird, and six were seen in a similar preparation made after 27 hr. The next day (2 days after the inoculation) the infection reached its peak (twenty-three flagellates being seen in a fresh preparation of blood $\frac{1}{2}$ in. square) and then slowly declined. An N.N.N. culture tube, inoculated with this rook's peripheral blood 45 min. after the injection of the flagellates, became positive, showing that a few trypanosomes must have been present in the bird's blood at that time, in spite of the failure to observe them. It was plain that other methods of observing the flagellates during the first 20 hr. or so of the infection would have to be devised.

Accordingly, canaries were given intraperitoneal inoculations of large numbers of metacyclic trypanosomes and were then sacrificed and autopsied at intervals.

At the first of these attempts, over 100,000,000 metacyclic trypanosomes (from 98 *O. avicularia*) were inoculated into a canary. Fresh preparations of blood from this canary were examined hourly from 2 to 6 hr. after inoculation, and a culture tube was inoculated with the bird's blood at 6 hr. also, but all these examinations were negative. The bird was killed 6 hr. after its inoculation and autopsied: fresh saline macerations and dried, stained impression smears were made from pieces of liver, lung, spleen, intestinal wall, kidney, ovary, wall of heart, bone-marrow and brain, and then examined. Fresh and dried smears of heart blood and peritoneal fluid were also examined, as was also a dried preparation made from the teased-up wall of the crop. In none of these preparations were any trypanosomes seen.

A second attempt was made, using two canaries (one as a control to the infectivity of the inoculum). The experimental canary was given about five times as large an inoculum as the control. It had been intended to kill and examine the experimental bird 16 hr. later, but it died overnight. At autopsy the following morning, it was examined similarly to the '6 hr.' canary referred to above: no flagellates were found. The control bird became infected.

Three similar experiments were conducted and all were successful. In these experiments, calculation of the number of metacyclic trypanosomes inoculated

was not possible because the dried films of the inoculum made for this purpose were poorly stained. In the first successful experiment, the canary (G 25) was killed 16 hr. after inoculation; in the second, the canary (G 26) was killed after 12 hr., and in the third, the canary (G 29) was killed only 6 hr. after it had been inoculated with flagellates. In each case fresh and dried microscopical preparations were made from, and N.N.N. culture tubes were inoculated with, pieces of various internal organs. The results of these examinations are set out in Table 1, and their significance is discussed below (p. 350). Examples of the trypanosomes found at these three periods after inoculation (together with some metacyclic trypanosomes for comparison) are shown in Fig. 2, and their measurements are recorded in Table 2. None of the trypanosomes seen during these investigations was undergoing division.

Table 1. *Details of examinations of canaries at autopsy for early developmental stages of* Trypanosoma avium

Number of canary	Hours after inoculation, when examined	Tissues examined											
		Bone marrow	Brain	Heart muscle	Heart blood	Intestinal caeca*	Kidney	Liver	Lung	Mesentery	Peripheral blood	Peritoneal fluid	Splee
G 25	16	+++	C	C	NX	++	+	C	+	+++	C	NX	+
G 26	12	++	NX	−	NX	+++	+	C	++	+++	++	++	C
G 29	6	−	NX	−	−	+++	−	−	+	+++	C	+++	-

Explanation of symbols: C, flagellates detected by culturing only; +, few, ++, intermediate number of, +++ many trypanosomes seen in preparations; −, no trypanosomes detected; NX, not examined.

* These organs were examined as a convenient source of lymphoid tissue.

In the first two of these experiments, a large inoculum was given to the experimental bird and a small amount of the same material was given to the control bird. In both cases the control canaries developed an infection. In the course of the third of these experiments, however, both canaries (G 28 and G 29) received equal inocula and were examined hourly after inoculation by means of fresh blood preparations about every 2 hr. (G 28 at 3, 5 and 7½ hr., and G 29 at 2, 4 and 6 hr.). No trypanosomes were seen in any of these preparations. One culture tube was inoculated with blood from G 28 at 3 hr. and remained negative. A tube inoculated with the peripheral blood of G 29 at 6 hr. became positive: in view of this, it is interesting to note that an uncontaminated heart blood culture made from the same canary at autopsy (immediately after the inoculation of the above tube) remained negative.

Trypanosomes having been found in the macerated intestinal caeca of G 29 (see Table 1). The other canary (G 28) was killed and autopsied 7½ hr. after it received its inoculation, the region of intestine bearing the caeca was removed, fixed in Carnoy's fluid, embedded and sectioned at 8 μ, in the hope that the exact region which the trypanosomes inhabited in these caeca could be determined.

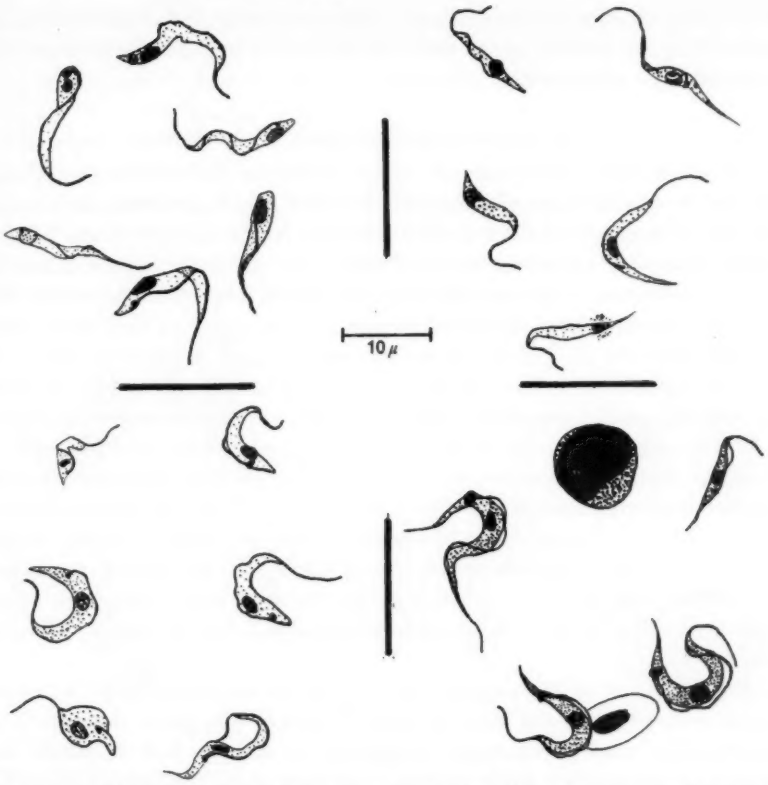

Fig. 2. Camera lucida drawings of *T. avium* during early stages of experimental infections in canaries, and metacyclic forms for comparison. Dried, Giemsa-stained preparations. Top left: metacyclic trypanosomes from smears of teased hind-guts of *O. avicularia*. Top right: trypanosomes from peritoneal fluid of a canary, 6 hr. after its inoculation (*i.p.*) with metacyclic forms. Bottom left: trypanosomes from teased mesentery of a canary, 12 hr. after inoculation. Bottom right: trypanosomes in bone-marrow (tibio-tarsal) of a canary 16 hr. after inoculation.

Table 2. *Average body lengths (excluding free flagellum) of metacyclic trypanosomes and five different developmental stages in canaries*

(Measurements in μ; flagellates from dried stained smears in each case)

Stage	Length of body		Number of flagellates measured
	Average	Range	
From insect			
Metacyclic trypanosomes	13·5	10·0–16·2	6
From bird			
6 hr.	12·8	9·3–17·0	6
12 hr.	13·8	6·3–17·3	6
16 hr.	19·0	12·3–25·7	6
24¼ hr.	28·6	25·0–30·5	3
ca. 72 hr.	47·7	34·9–54·3	25

However, examination of the sections, while confirming the existence of a large amount of lymphoid tissue in the walls of the caeca, has so far failed to reveal the presence of any trypanosomes.

(*b*) *Development in* Ornithomyia avicularia

The developmental cycle which *T. avium* undergoes in its vector was followed chiefly by the examination of preparations of the teased-up alimentary canals of insects, made at varying intervals after they had fed on infected birds. The louse-flies used in these experiments can be divided into two main groups, A and B.

Group A consisted of flies which were allowed to feed on a naturally-infected rook from a feeding box (described in the second paper of this series, Baker, 1956*b*) for about 24 hr. Trials had shown that this rook's infection was so weak that flies which fed on it once only did not develop an infection. After this period on the infected rook, some of the flies were removed and maintained on a 'clean' bird: others were left on the infected bird. Thirteen insects of this group were dissected as controls, and none was found to be harbouring a natural infection of flagellates: similarly, none of nine flies dissected 1 week earlier had been infected. However, nine out of twenty-one dissected 1 week later were naturally infected.

The insects in this group were subsequently killed and examined at the following intervals after they were first placed on the infected rook: three flies killed at 2 days, then one fly daily from the third to the eighth day inclusive. All of these flies were infected.

Group B consisted of flies obtained late in the season, when some of the 'wild' *O. avicularia* were naturally infected. Individuals of this group were all infected or reinfected by single feeds (from glass vials) on canaries with relatively heavy experimental infections. After feeding, the flies were maintained in a moist atmosphere at 30° C. and then killed and examined after the following intervals: 15, 30, and 45 min.; 1, 1½, 2, 2½, 3, 4, 6, 24 and 72 hr. One insect was examined at each of these times, except at 30 min. and 1 hr., when two were dissected: all were infected. The timing of the development occurring in these insects is probably somewhat slower than that occurring in nature, as the flies were maintained at a lower temperature than the average skin temperature of rooks, which was subsequently determined by means of thermocouple readings* as about 39–40° C.

Preparations made from insects of group B were used to obtain a picture of the early stages of the flagellate's development, the later stages being interpreted from preparations made from both groups.

(i) *Development in the mid-gut*

The large trypanosomes ingested with the louse-fly's blood meal change quite rapidly into crithidia. The intermediate forms which have been found in the mid-gut suggest that this change occurs, generally, by a rolling up, or folding back, of the trypanosome's elongated posterior extremity, followed by the break-down of the contiguous regions of the surface membrane (see Fig. 3*a*, *b* and Fig. 4*a*).

* I am indebted to Mr M. K. Totton for taking these readings.

This results in the formation of a large, 'blunt-nosed' crithidia (Fig. 4*b*). There is some evidence that this initial transformation from trypanosomes to crithidia may, in some cases, occur by simple resorption of the posterior extremity (see Fig. 3*c*).

These large crithidia, which have been seen in the mid-guts of louse-flies of group B killed 1 hr. after feeding, then commence binary division. By 2 hr. (at 30° C.) the products of this first division (crithidia, see Fig. 4*c*) are themselves beginning to divide (Fig. 3*d*, *e*). This division gives rise to smaller, somewhat spindle-shaped crithidia (Fig. 4*d*), which have been seen in mid-gut contents of insects $2\frac{1}{2}$ hr. after feeding. The spindle-shaped crithidia then divide by binary

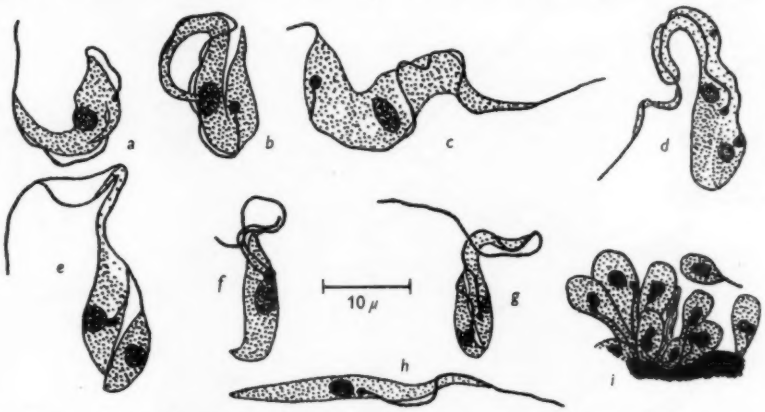

Fig. 3. Camera lucida drawings of *T. avium* from the mid-guts (except *i*) of *O. avicularia*, supplementary to those shown in Fig. 4. (All from wet-fixed, Giemsa-stained preparations: the times given below refer to the interval (at 30° C.) between ingestion of the trypanosomes by the louse-fly, and the dissection of the latter.) *a*, trypanosome, 15 min.; *b*, trypanosome, 45 min.; *c*, trypanosome, 30 min.; *d*, dividing crithidia of second generation, 2 hr.; *e*, dividing crithidia, division more advanced, $2\frac{1}{2}$ hr.; *f*, dividing third-generation crithidia, $2\frac{1}{2}$ hr.; *g*, dividing third-generation crithidia, 4 hr.; *h*, later crithidia, 6 hr.; *i*, group of haptomonad crithidia from hind-gut, natural infection.

fission (see Fig. 3*f*), and the products of this division give rise, by growth, to the rather more elongated but still fairly stout crithidia seen in preparations made from louse-flies 4 hr. after their infective feeds (Fig. 4*e*). These latter crithidia undergo yet another (the fourth) binary division (Fig. 3*g*), the products of which are assumed to grow into the large elongated crithidia seen 24 hr. after ingestion by the insect (Fig. 4*f*), via stages like that shown in Fig. 3*h* (6 hr.). As no preparations were made between 6 and 24 hr., it is impossible to state whether or not further binary divisions occur between the stage shown in Fig. 3*h* and that shown in Fig. 4*f*. It seems probable that further divisions do occur here.

Large crithidia of the type shown in Fig. 4*f* were found in the mid-guts of two flies of group A which had been maintained on an uninfected rook for 4–5 and 7–8 days, respectively, after their infective feeds. In one fly maintained on a clean bird for 2–3 days after its infection, however, no flagellates were seen in the mid-gut, although many were present in the hind-gut. In order to account for the

apparent persistence (in some cases) of the long crithidia, the cycle shown by dotted lines in Fig. 4 has been postulated. The forms shown in this cycle (Fig. 4*f-h*) were all found in the mid-guts of louse-flies of group B killed 24 hr. after their

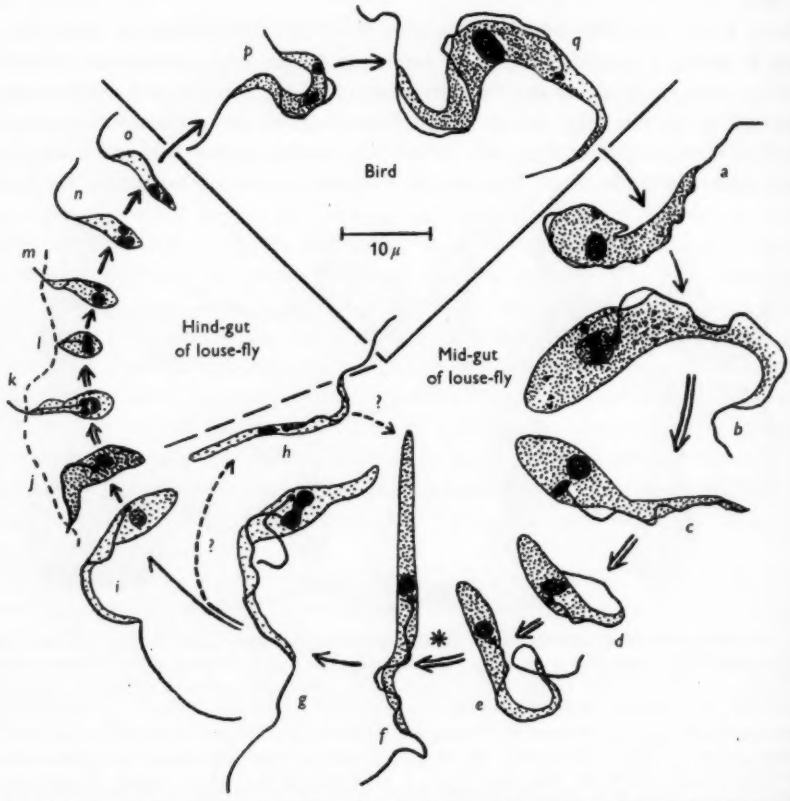

Fig. 4. Scheme of development of *T. avium* in bird and louse-fly. Camera lucida drawings to the same scale. Double arrows indicate division. At the point marked with an asterisk (*), division probably occurs more than once. The dotted line on the left of the figure represents the peritrophic membrane. *a–o*, wet-fixed Giesma-stained preparations from flies; *a–j*, from mid-gut (times refer to interval (at 30° C.) between infection and dissection of louse-flies); *a*, trypanosome, 30 min.; *b*, dividing crithidia, 1 hr.; *c*, product of 1st division, 1½ hr.; *d*, product of 2nd division, 2½ hr.; *e*, product of 3rd division, 4 hr.; *f–i*, various nectomonad crithidial forms, 24 hr.; *j*, ? haptomonad crithidia, 24 hr. *k–o*, from hind-gut (*l–o*, natural infections); *k*, dividing haptomonad crithidia (2nd generation); *l*, *m*, ? haptomonad (pyriform) crithidia; *n*, nectomonad crithidia (pre-metacyclic form); *o*, metacyclic trypanosome. *p*, *q*, from dried Giesma-stained films of bone-marrow and peripheral blood, respectively, of canaries; *p*, partially grown trypanosome, 16 hr. after inoculation; *q*, fully grown trypanosome, 3 days after inoculation.

infective feeds. It is suggested that the long crithidia (*f*) undergo unequal binary fission (*g*) to produce broad crithidia (*i*) which continue the main development, and narrow individuals (*h*) which grow into the large forms again, and so repeat the cycle.

Flagellates have not been seen in serial sections of the mid-guts of infected flies, so it is not possible to state decisively whether or not these 'mid-gut forms' are attached to the gut wall or free-swimming. Their structure, and their behaviour in fresh teased preparations, suggest that they are free (nectomonad) forms. Serial sections have shown beyond doubt that the majority of the forms found in the hind-guts of infected flies are attached to the peritrophic membrane (haptomonad forms, see Fig. 5). The broad crithidia (Fig. 4*i*) found 24 hr. after ingestion (at 30° C.) are thought to attach themselves to the peritrophic membrane of either the extreme posterior region of the mid-gut, or the anterior region of the hind-gut. The form shown in Fig. 4*j* may represent an early attached phase, or a transition form between this and the nectomonad individuals.

Fig. 5. Photomicrograph of part of a section (at 5 μ) through the rectum of an infected louse-fly, to show the (mainly haptomonad) flagellates. Giemsa-colophonium staining. × 600.

The early development in the mid-gut is not synchronous, some flagellates with the trypanosome structure being found at 1 hr. after ingestion, while in the same preparation crithidia in the early stages of division can be seen. This asynchronicity might be due to the fact that the insects were kept at a temperature probably nearly 10° C. lower than that of their natural habitat (see p. 346).

(ii) *Development in the hind-gut*

As has just been stated, the majority of the forms found in the hind-gut are attached to the peritrophic membrane. This attachment is by their anterior (flagellar) ends. However, some narrow crithidia (both short and long), of the type shown in Fig. 4*f*, *h*, have been seen in the hind-guts of infected flies; these are presumably free-swimming forms. Also, it seems likely that the metacyclic trypanosomes themselves are free-swimming. In the sections which have been examined it is not possible to ascertain the structure of most of the flagellates present: this has been done from smears of the contents of the hind-gut.

The large haptomonad or pre-haptomonad forms mentioned above (Fig. 4*j*) undergo division (probably at least twice), giving rise eventually to small pyriform attached crithidia, which are found throughout the hind-gut (see Fig. 4*k-m*). A group of these pyriform crithidia of various sizes is shown in Fig. 3*i*: this group was found on a smear preparation made from the colon of a naturally infected fly, and demonstrates clearly the attachment of the flagellates by their anterior ends.

The small pyriform crithidia probably become free-swimming again (although this is not certain), and become more elongated, giving rise to the small crithidia shown in Fig. 4*n*. These 'pre-metacyclic' forms, by a posterior migration of the kinetoplast, change into the infective metacyclic trypanosomes (one of which is shown in Fig. 4*o*), which are found throughout the hind-gut, and are not restricted to the rectum. A few rounded leishmaniform individuals were sometimes seen in preparations of hind-gut contents. The significance of these forms is not known: none showed any signs of encystment. Some idea of the density of the infection built up may be gained from Fig. 5, which shows flagellates attached to a part of the wall of the rectum of a louse-fly.

The hind-gut phase of development commences at about 48 hr., or perhaps a little earlier, after the ingestion of trypanosomes by the louse-fly, and metacyclic trypanosomes first appear at about 60 or 72 hr.

The course of development observed in naturally infected louse-flies does not appear to differ from that described above.

V. DISCUSSION

It is remarkable that, in the foregoing work, no trypanosomes were seen to be undergoing division while in the bird. The evidence suggests strongly that, in canaries at any rate, the metacyclic trypanosomes do not multiply after being introduced into the bird. Instead, they proceed to the lymph system, where they grow. The majority enter the peripheral blood system between 18 and 24 hr. later. In heavy experimental infections, however, it is clear that some do this earlier. Also, study of the graphs of Fig. 1 shows that some trypanosomes do not enter the peripheral circulation until 3 or 4 days after their inoculation. These observations were, of course, made on unnatural hosts which had been infected by unnatural routes. However, such evidence as is available from infections produced in wild birds (rooks and a thrush) by their ingestion of louse-flies offers no foundation for the belief that the behaviour of the parasite under these conditions is different.

There is one difference between infections in canaries, and those in the natural hosts: in the latter, the infection survives the winter in the bone-marrow of the bird (as was shown earlier by Minchin & Woodcock, 1912, and others), whereas in canaries the infection is apparently of shorter duration, the flagellates not persisting (at any rate for long) in the bone-marrow after they have left the peripheral blood. As trypanosomes in the bone-marrow of birds were so scanty, it is impossible to say whether or not some multiplication occurs during the winter: if it does not (and no evidence for it was obtained in this work), either (*a*) some other vector must participate in transmission between November and May, when adult *Ornithomyia avicularia* are absent from this country, or (*b*) individual

parasites must persist in the host for some 4–5 months. Evidence against the operation of another vector during this period is twofold: (i) the at least partial restriction of the trypanosomes to the bone-marrow; and (ii) the general epidemiology of the infection. In 1954, louse-flies were first found on birds at the end of May, and, by early July, some of the insects were naturally infected with flagellates. From mid-April to mid-August, 103 'first-year' rooks had been examined, and none was infected: the first infected 'first-year' bird was trapped during the third week of August. Thus it seems that no transmission occurred until flagellate infections had developed in the louse-flies.* It is impossible to decide between the two remaining possibilities (i.e. that individual trypanosomes survive for 4 or more months in the bird, or that division occurs throughout the winter at a low rate) without further evidence. Concerning *Trypanosoma melophagium* of sheep (a parasite similar in appearance to *T. avium*, which undergoes a somewhat similar development in a related insect), Hoare (1923) wrote 'it is conceivable that the trypanosomes introduced into the sheep by the ked do not multiply at all in the former'.

Concerning the development of the flagellate in the louse-fly, little need be said. The scheme of development outlined above (pp. 346–350) is only provisional, and does not claim to be complete in every detail. It is worth stressing that, during this work, no trace of any sexual process or intracellular stage of the trypanosome has been observed, in either the bird or the insect.

VI. SUMMARY

1. The life cycle of *Trypanosoma avium* in both its hosts is described.

2. In the vector (*Ornithomyia avicularia*) it undergoes a cyclical development in the alimentary canal. Multiplication occurs while the flagellate is in the crithidial stage, and, finally, pyriform haptomonad crithidia appear in the insect's hind-gut: these change into the infective metacyclic trypanosomes.

3. After penetrating the mucous membranes of the bird (following its ingestion of the louse-fly), the metacyclic trypanosomes probably invade the lymph system, and there develop (by growth alone) into the large forms seen in the bird's blood some 18–24 hr. later.

4. The trypanosomes persist in their natural hosts (but not in canaries) throughout the winter, more-or-less restricted to the bone-marrow. In the spring they reappear in the peripheral blood.

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* An overall percentage of 30·4 of these insects, out of 112 dissected from May to September 1954, had natural infections of flagellates.

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(*MS. received for publication* 18. VI. 1955.—Ed.)

ON THE GENUS *TRIPSELIA* AND THE GROUP OF *BASILIA BATHYBOTHYRA* (NYCTERIBIIDAE, DIPTERA)

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(With 57 Figures in the Text)

Some species of Nycteribiidae have been recorded from widely different faunistic regions. Thus, *Eucampsipoda hyrtlii* has been recorded from Africa, Ceylon, Burma and Sumatra. Other species have been recorded from Europe and China or from Europe and South Africa. Re-examination of the material has proved in most cases that the species were wrongly identified and no species has been found so far to exist both in Europe and in East Asia. There remained finally two species which have been recorded from the Ethiopian and the Oriental region, *Tripselia blainvillii* and *Basilia bathybothyra*. Material of these two species from as many localities as possible was therefore brought together, in order to determine whether this wide distribution does in fact occur or whether, in these cases as well, there existed different forms, species or subspecies, with a narrower range of distribution.

The material available for examination consisted of over 70 specimens of *Tripselia blainvillii* (either identified as *T. amiculata* or unidentified), of new species of *Tripselia* from Borneo, Sumatra and New Guinea and of about 150 specimens of '*Basilia bathybothyra*' from the Eastern Mediterranean, Africa, India and Indonesia. In addition, a number of specimens from Australia were examined, which also proved to belong to the genus *Tripselia*. Of these only the specimens of *Tripselia falcozi* were identified and will be included in the present paper. Some of the others have probably been described by Musgrave as species of *Nycteribia*, others are obviously new. There is, however, too little material available at present to deal with these forms.

I have to thank the authorities of the British Museum (Nat. Hist.), Dr R. Wenzel of the Natural History Museum, Chicago, Dr J. Bequaert of the Museum of Comparative Zoology, Cambridge, U.S.A., Dr V. Aellen of the Muséum d'Histoire Naturelle, Geneva, Dr P. Basilewski of the Musée du Congo Belge, Tervueren, Dr E. Séguy of the Muséum d'Histoire Naturelle, Paris, and the Director, Indian Museum, Calcutta, for placing their material at my disposal.

The material in the British Museum included the collection made by Dr H. Scott and the Rothschild collection, both of which contained very rich and interesting material.

Genus **Tripselia** Scott, 1917

Type species: **Tripselia blainvillii** Leach, 1817

The genus was named by Scott (1917) on the basis of a letter by Speiser who called Scott's attention to the absence of eyes in *T. amiculata* and proposed the name *Tripselia* because of the presence of three rings on the tibiae, which had

induced him originally to include the species in the genus *Cyclopodia*. Scott's definition of the genus is rather short and characters which have proved specific for *Tripselia blainvillii*, e.g. the very long legs, are considered as generic characters.

A provisional diagnosis of the genus is as follows: Closely related to the genus *Basilia*, differing from it mainly in the absence of eyes. Head laterally compressed, as in the genus *Nycteribia*. Eyes absent. Thorax as in the genera *Nycteribia* and *Basilia*. Halter grooves open. Legs either very long or of normal length. Tibiae with 3 rows of setae on the ventral surface. Tergite 1 of the abdomen of the female with two groups of long setae posteriorly. Tergal plate 2 heart-shaped, incompletely divided in the middle, with posterior processes which bear long setae. Tergal plate 3 with some setae. Abdominal ctenidium well developed. Post-spiracular plate as in *Basilia*. Sternite 5 and 6 divided into two plates. Dorsal genital plate as in *Basilia*, consisting of an area covered with minute hairs and bearing several long setae. Anal sclerite present, bearing setae. Adanal plates present.

Abdomen of male with seven tergites which bear long setae posteriorly and groups of short hairs on the surface. Sternite 5 with an armature of short thick spines at the posterior margin. Male genitalia as in *Basilia*. Aedeagus tapering to a point, either straight or curved.

On the whole, this definition applies equally well to some species of the genus *Basilia*, particularly the group related to *Basilia bathybothyra* and the question arises whether the absence of eyes should be considered as a generic character. Degeneration of eyes has apparently occurred simultaneously in various groups. Absence or presence of eyes is certainly a convenient character to use in classification, but the similarity between the genus *Tripselia* and some species of *Basilia* is so marked that one is tempted to consider *Tripselia* as a subgenus of *Basilia*.

(1) **Tripselia blainvillii blainvillii** Leach, 1817

Syn. *Nycteribia fryeri* Scott, 1914.
Tripselia fryeri Scott, 1917.
Tripselia amiculata Speiser, 1907 apud Scott, 1925 pro parte.
Nycteribia reichenowi Karaman, 1948.
nec *Nycteribia blainvillii* Leach apud Speiser, 1901.

Phthiridium blainvillii was described by Leach (1817) as follows: Ph. pedibus longibus tenuibus. Femoribus articulo 2 tibiisque articulo 1 apicem versus gradatim attenuatis. Long. corp. $1\frac{3}{4}$ lin. Habitat in Insula Isle de France.

Westwood (1835) examined Leach's specimen in the British Museum and added to Leach's description: obscure ochraceo-livida, abdomine (apice excepto) fusco, elongato conico, depresso, segmentis sex apice setigeris, ultimo longiori subrotundato (♂) Long. corp. lin. 1. ($1\frac{3}{4}$ secundum Leach). Mus. Brit.

Speiser (1901) described *Nycteribia blainvillii* from Egypt. His material, however, proved to be different from the species described by Leach from Mauritius and the species has been re-named *Nycteribia integra* by Theodor and Moscona (1954). In 1907, Speiser described *Cyclopodia amiculata* found on *Taphozous longimanus* in Calcutta.

Scott (1914) described *Nycteribia fryeri* from the Assumption Islands and from Labuan. In 1917 he considered this species as probably identical with Speiser's *Cyclopodia amiculata* and adopted Speiser's proposed generic name *Tripselia*. This view was confirmed by him in 1925 after comparison of his specimens with Speiser's type. Speiser's species is not identical with the form from the Assumption Islands and Mauritius and is considered as a subspecies of *Tripselia blainvillii*.

In 1948 Karaman described *Nycteribia reichenowi* from East Africa from *Taphozous mauritianus*. This also proved identical with *Tripselia blainvillii* on examination of the types.

Comparison of the types of *T. fryeri* with Leach's type of *Phthiridium blainvillii* in the British Museum proved them to be identical, so that the correct name of the species is *Tripselia blainvillii* Leach, 1817.

The following description is based on paratypes of *T. fryeri* from the Assumption Islands, material from Africa and the specimen from Labuan examined by Scott. This description is given with full details. In the following descriptions mainly details which differ from those of *T. blainvillii* will be given. Measurements of the thorax are made at the sternal plate. Length is measured in the median line and width at the widest part posteriorly.

Description. Length 2·5 mm. Colour light brown.

Head laterally compressed. The anterior dorsal margin forms an angle with the apex posteriorly. Along each side of the angle three setae. Short setae along the anterior ventral margin of the head with a longer seta posteriorly. Five to six short setae on the genae. Palps with a very long terminal seta, a shorter seta on the ventral surface near the tip and four to five short setae at each side which become shorter towards the base. Labella of the labium as long as the theca or slightly shorter. Theca rather narrow.

Thorax. Ventral plate rounded anteriorly, slightly wider than long. Median ventral suture indistinct in middle. Oblique sutures forming an angle of about 90°. A uniform row of very short hairs along the posterior margin of the sternal plate, as long as those on the surface. Mesonotum moderately wide, rounded posteriorly. Lateral plates of the notopleural sutures wide, with eight to eleven notopleural setae. Thoracic ctenidia with about eighteen pointed spines. Legs very long and slender, particularly the hind legs. (3·5 to 4 mm.) (Fig. 1.) Tibiae very slender, about eight times as long as wide, with three rows of setae near the middle of the ventral surface. These setae are comparatively short, those of the distal row just reaching the tip of the tibiae, those of the middle row about half as long and those of the proximal row still shorter. As these setae are short and few in number, the stripe of weak chitin distal to the rows stands out more clearly than in other species with many thick setae. There is one very long seta near the base and two in the apical half of the dorsal surface of the tibia.

Abdomen female (Figs. 2 and 3). Tergite 1 forms two broad lobes posteriorly each of which bears about five long setae, the median setae being as long as the more lateral ones, and a few shorter ones at the sides. Two oblique groups of short setae on the surface of the tergite which are shorter and thinner basally

and longer posteriorly. Two pigmented stripes along the posterior margin of the lobes and then running across the tergite towards the base. Tergal plate 2 large, heart-shaped, divided in the middle. This is probably formed by tergites 2 and 3, judging from the position of the spiracles. There are two rather narrow, pointed posterior processes which bear a very long seta, a shorter one at the tip and four to five short spines. Two pigmented stripes run from the posterior processes into the middle of the lateral halves of the tergal plate, parallel to the median line, through about half of the length of the tergal plate. Three to five moderately long, widely spaced setae along the posterior lateral margins. Short setae on the

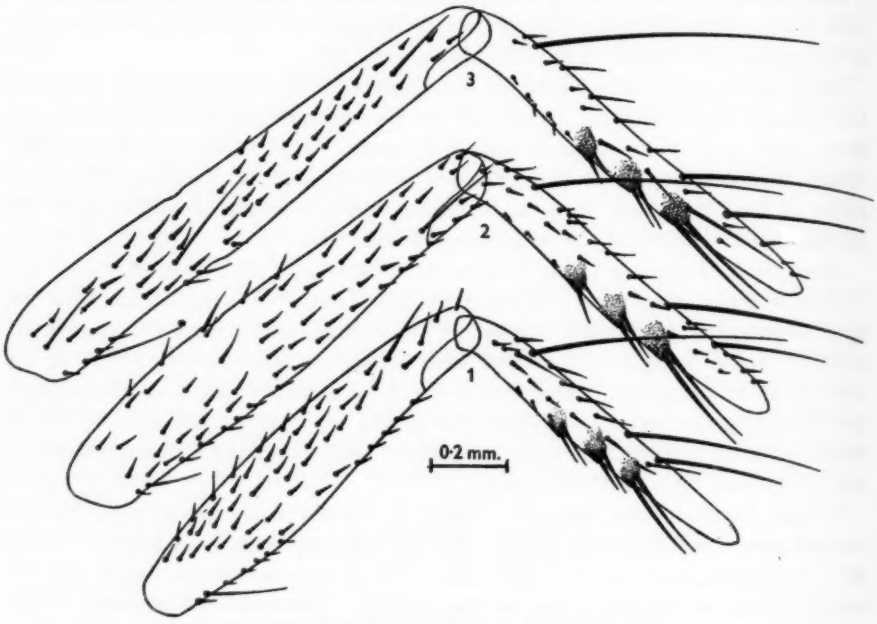
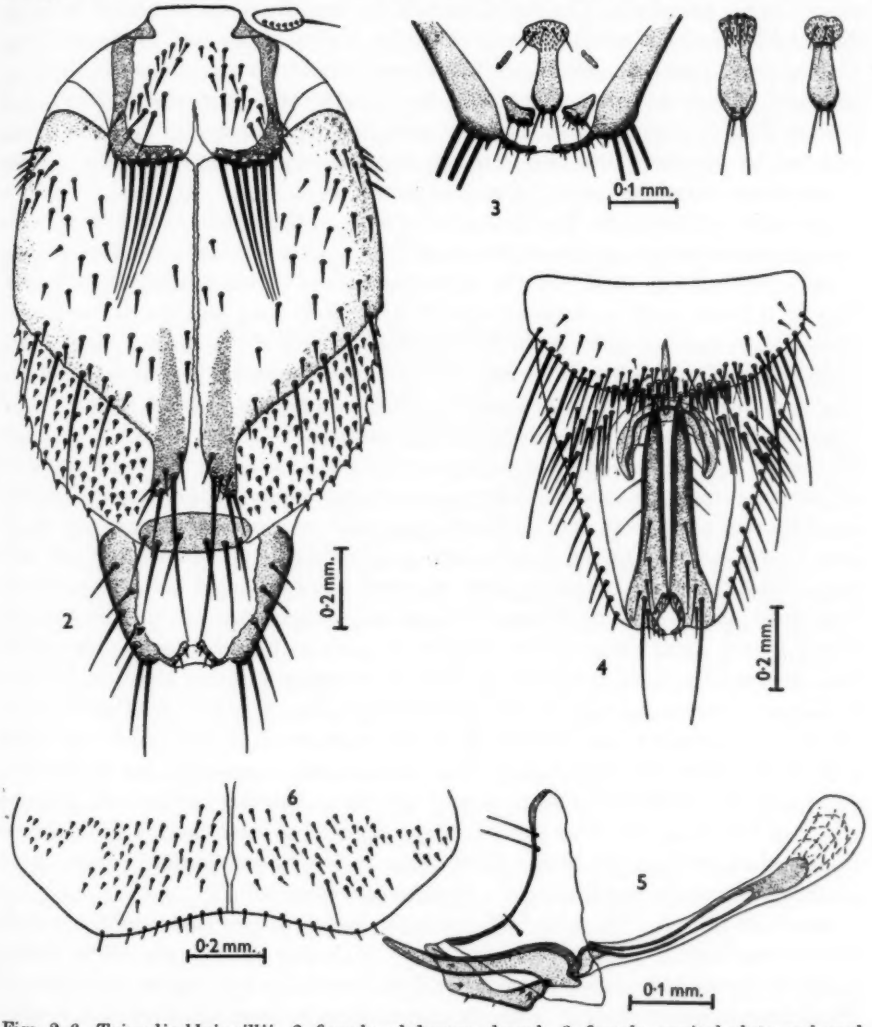


Fig. 1. *Tripselia blainvillii*, legs (femur and tibia).

surface of the plate, mainly at the sides. A few such setae along the median line. Pleurae covered with minute thick spines. Tergal plate 3 narrow, transversely elliptical, with four to eight moderately long setae near the posterior margin. Spiracles 5 to 7 close together, rather far posteriorly. Anal segment short, truncate, sclerotized laterally, bare dorsally, with some short setae laterally and two to three long setae and some short spines at the short anal processes. Sternite 1 + 2 rounded anteriorly with a ctenidium of fifty to fifty-five long teeth and about four rows of short setae in the posterior part of the surface. These setae are longer and thicker laterally. Postspiracular plate broad with one or two longer setae towards the spiracle and a row of six to seven minute spines along the posterior margin. Sternite 3 rather wide with concave posterior margin which bears a uniform row of moderately long setae and five to six rows of short setae on the surface. Four to six setae which are longer and thinner than those of the marginal

row in the posterior row of the surface. Sternite 4 narrow, with a similar marginal row and a single row of short setae on the surface. Sternite 5 with two narrow, widely separated sclerites at the sides. The seven or eight lateral setae of the marginal row stand on these sclerites and four to six setae between them. One or



Figs. 2–6. *Tripselia blainvillii*. 2, female, abdomen, dorsal. 3, female, genital plate and anal sclerites. 4, male, sternite 5 and genital area. 5, male, genitalia. 6, posterior margin of sternal plate of thorax.

two of the setae at the sides are much longer than the rest. Sternite 6 with two broader, semicircular plates with straight posterior margin which do not quite reach the median line. Two setae between them in some specimens, none in others. A row of long setae at the posterior margin of which one or two at the sides are

longer than the others and a transverse row of short setae on the surface. Sternite 7 triangular or rounded posteriorly with a row of long setae in the middle of the posterior margin which become gradually shorter towards the middle. A row of four long setae on the surface near the marginal row and a transverse row of short setae further anteriorly. The dorsal part of the anal frame is connected with the lateral sclerite of the anal segment. Ventrally, a part of the anal frame with four to five setae has been separated and forms adanal plates. Between them an elliptical sclerite with two longer and two shorter setae posteriorly. The dorsal genital plate is a transversely elliptical area covered with minute hairs and with an irregular transverse row of four to six setae across the middle. The anal sclerite is sometimes connected with the genital plate.

Abdomen male (Fig. 4). Tergite 1 shorter than in the female with slightly concave posterior margin which carries six to eight short setae at each side, leaving a wide gap between them. Six to eight short setae on the surface in each half. Tergite 2 broad, with a marginal row of moderately long and shorter setae and two to three rows of short setae in the posterior part of the surface which reach to the sides. Tergites 3 to 6 narrow with similar marginal rows, but with two to four very long and thick setae near the middle on tergite 5 and 6. Two to three rows of short setae in the middle of the surface of tergite 3 and 4, one row on tergite 5, tergite 6 bare. Anal segment about as long as the four preceding segments, conical, with some longer and shorter setae on the dorsum, mainly laterally and posteriorly and a row of longer and shorter setae posteriorly. Sternite 1 + 2 similar as in the female with a ctenidium of forty to fifty very long teeth. Sternite 3 and 4 narrow with marginal rows of longer and shorter setae. Two setae at the sides of sternite 4 much longer than the others. Two to three rows of very short setae on the surface of sternite 3 and a single row, which may be broadly interrupted, on sternite 4. Sternite 5 much broader, rounded posteriorly. In the middle of the posterior margin a double row of short, thick spines. The posterior row consists of twelve to fourteen spines which are longer and more widely spaced laterally. The anterior row consists of ten to fourteen shorter spines which stand more closely together. Anterior to the rows of spines a row of four long and some shorter setae. Some long setae laterally to the rows of spines at the margin. Some short setae in the lateral posterior part of the surface, close to the marginal row. Surface otherwise bare.

Genitalia (Fig. 5). Claspers long and thin, darkly pigmented, slightly curved. A long seta dorsally near the base and a row of shorter setae on the dorsal surface to about the middle of the clasper. Basal arc rounded with long anterior process. Phallobase concave dorsally with two pairs of setae near the base and another pair distally. Aedeagus uniformly tapering to a point, slightly curved. Apodeme with a wide end-plate. Parameres with blunt tip and an obtuse, rounded ventral angle. A longer hair in the distal part and some minute hairs at the ventral edge and near the base.

Distribution. Mauritius, Assumption Islands. Tropical Africa from West to East between 10° latitude north and south.

Hosts. Mainly *Taphozous mauritianus.*

Material examined. Type in the British Museum. (1 ♂) *Mauritius* (Leach). *Assumption Islands.* (1 ♂, 5 ♀) *Taphozous mauritianus.* (Fryer.) (Paratypes of *T. fryeri* Scott). *West Africa: Sierra Leone,* Palmaro. (2 ♂, 1 ♀) *T. mauritianus.* (Bequaert.) Njala. (1 ♀) *T. mauritianus.* (Jones.) *Gold Coast,* Accra (1 ♂, 2 ♀) 'grey house bat'. (Bromer.) (1 ♂) (Macfie). *Dahomey,* Agoumgoum from *Rousettus* sp. (1 ♀) (Bouet). *Cameroons,* Kribi (3 ♂, 2 ♀) *T. mauritianus.* (Aellen.) *North East Angola,* Dundo, Lunda. (2 ♂, 4 ♀) *T. mauritianus.* (Barros Machado.) *Belgian Congo,* Bambesa. (1 ♂, 1 ♀) *T. mauritianus.* (Vrydagh.) Ituri forest (2 ♂, 2 ♀) (Lord Howard de Walden Expedition). *East Africa: Anglo Egyptian Sudan,* Opari, Torit. (6 ♂, 8 ♀) *T. mauritianus.* (Hoogstraal.) Li Rangu, Equatoria. *T. mauritianus.* (2 ♂, 2 ♀) (Alison). Mundiri, Equatoria prov. *T. mauritianus.* (4 ♂, 5 ♀). (Owen.) *Kenya,* Kyambu. (3 ♀) (Garnham). *Tanganyika,* Ujiji. (2 ♂) *Epomophorus anurus.* (Loveridge.) Kilossa. (1 ♀) *T. mauritianus.* (Loveridge, Rothschild Collection.)

(2) **Tripselia blainvillii amiculata** Speiser, 1907

Syn. *Cyclopodia amiculata* Speiser, 1907.

One female and two males from Calcutta, one male and one female from Burma, three females from Ceylon, a female from Labuan and a male from Sumatra were available for examination. These specimens are slightly smaller and more slenderly built than the African specimens, but no marked difference from the typical form was found. The short setae on the surface of tergal plate 2 are less numerous. There are only two or three longer setae at the posterior lateral margin of tergal plate 2 and the long setae at the posterior processes are shorter. The abdominal ctenidium has only forty to forty-five teeth. The males also differ in some details. Tergite 2 has five to six rows of short setae covering nearly the whole surface, while there are only two to three rows in the posterior part of the surface in the typical form. The anterior row of spines on sternite 5 has ten to twelve spines in the males from Calcutta and Burma as in the typical form, but only six spines in the male from Sumatra. Sternite 5 is narrower and less curved posteriorly and the setae at the posterior margin and in front of the armature of spines are shorter. There is no difference in the genitalia.

The material is not sufficient to decide whether the specimens from Ceylon and Sumatra are identical with those from Calcutta, and whether the relatively small differences are constant. Provisionally the Indian form is considered as a subspecies of *T. blainvillii* and Speiser's name *amiculata* is used for it. The type of *T. amiculata* has been lost during the last war, as Dr Kapur of the Indian Museum informs me, and a female and a male from the collection of the Indian Museum (from the type locality and the type host) are designated as neotypes.

Material examined. Calcutta, *Taphozous longimanus.* (1 ♀) (Neotype). (Kemp.) Port Canning, Calcutta, *Taphozous* sp. (2 ♂) (Annandale) (Neotype and Paratype.) Padaung, Prome district. *Burma* (1 ♂, 1 ♀) (Jain).

Ceylon. Trincomalee. (1 ♀) *Saccolaimus saccolaimus.* (Phillips.) Gammaduwa, East Matale. (1 ♀) *Saccolaimus saccolaimus.* (Phillips.) Anasigalla, Matugama, Kalutara. (1 ♀) *Taphozous longimanus.* (Phillips.)

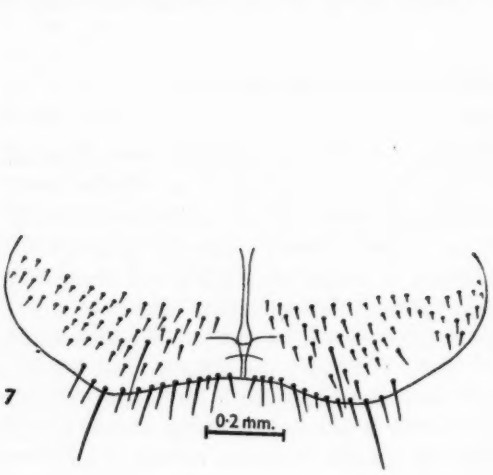
Sumatra, Balighe. 1 ♂. (Modigliani.) *Borneo*, Labuan. *Saccolaimus saccolaimus* (1 ♀).

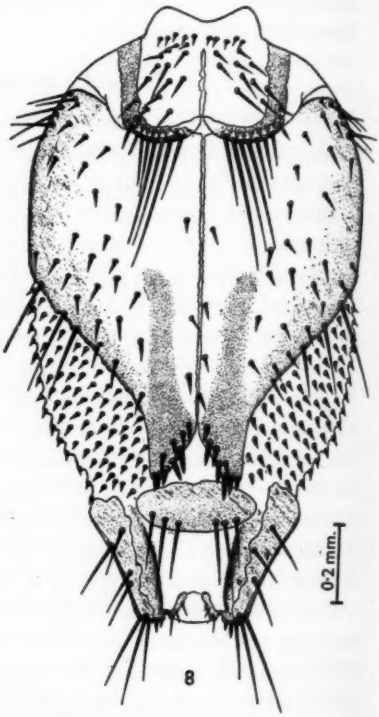
The female from Labuan has the setae of tergite 1 shorter and more widely spaced. It possibly belongs to the same form as the male of Sumatra and may prove eventually to be a separate subspecies.

(3) **Tripselia aequisetosa** n.sp.

This species resembles *T. blainvillii* closely and the males of both species are nearly indistinguishable.

Head and *thorax* as in *T. blainvillii*, except that the row of hairs at the posterior ventral margin of the thorax consists of some longer setae, of which one near the side is the longest, and some short ones between them (Figs. 6, 7). Legs as





Figs. 7, 8. *Tripselia aequisetosa* n.sp. 7, posterior margin of sternal plate of thorax. 8, female, abdomen, dorsal.

in *T. blainvillii*, but even longer and tibia 1 is relatively shorter in comparison to tibia 3. $T_1 : T_3 = 11 : 13$ in *T. blainvillii* and 11·5 : 15 in *T. aequisetosa*. The main differences are in the abdomen of the female (Fig. 8). There are only one to three long setae on tergite 1 and the innermost seta is about half as long as the next one. The lateral setae become gradually shorter so that the whole group appears triangular, while in *T. blainvillii* there are four to six long setae of about equal length including the innermost and the lateral ones are much shorter. The

posterior processes of tergal plate 2 are much broader than in *T. blainvillii* and bear no long setae, but only an irregular single or double row of six to nine short spines of about equal length. No difference was found in the males except in the row of setae at the posterior ventral margin of the thorax.

Material examined. Nigeria, Lagos. (3 ♀, 1 ♂) bat no. 18. (Lowe.) *Liberia*, Du River. (1 ♂) *Saccolaimus peli*. (Bequaert.) *Belgian Congo*, Medje. (1 ♀) *Saccolaimus peli*. (Bequaert.)

The distribution of *T. aequisetosa* thus coincides in part with that of *T. blainvillii*, but will probably prove wider when more material is available.

(4) **Tripselia brevipes** n.sp.

A form very similar to this species was mentioned by Scott (1917) and was later considered by Musgrave (1927) as closely related to *T. falcozi*.

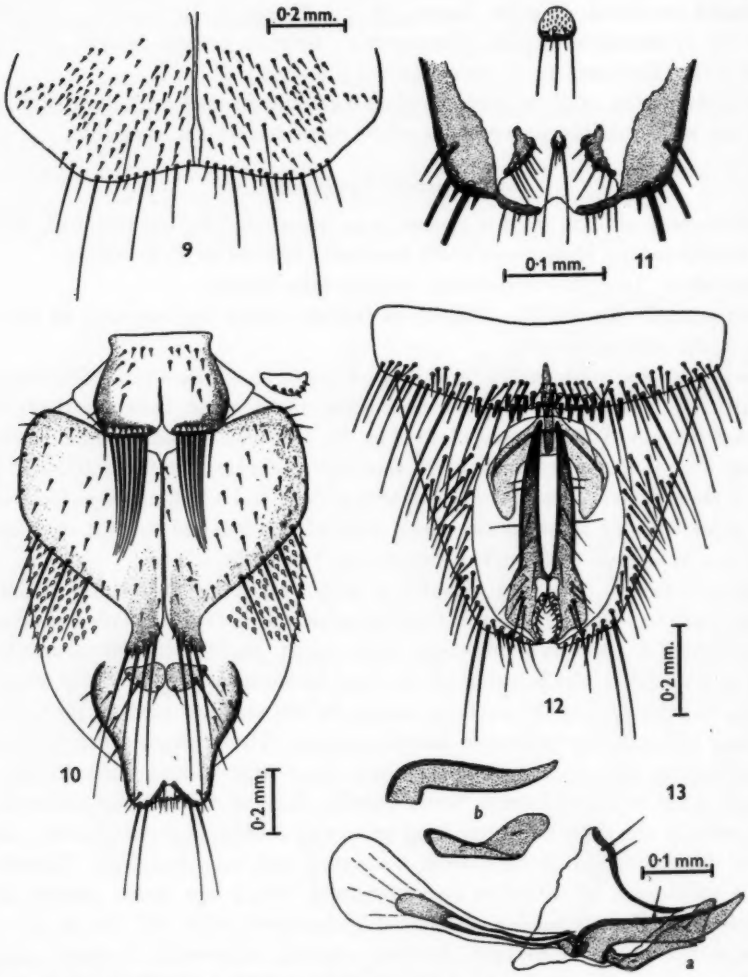
Description. Length 1·5 to 2 mm. Colour light brown.

Head as in *T. blainvillii*. Labella of labium about half as long as the theca which is elongate elliptical.

Thorax. Sternal plate wider than long. Length to width = 4 : 5. Six long setae, of which the lateral are longest, and some short setae between them at the posterior margin of the sternal plate (Fig. 9). Otherwise much as in *T. blainvillii*. The legs are much shorter than in *T. blainvillii*. Foreleg 2·5 mm. Hindleg 3 mm. long. Tibiae flattened, scalpel-shaped, four to four and a half times as long as wide, with three rows of setae in the distal half of the ventral surface of which the distal row is longest and reaches beyond the tip of the tibia.

Abdomen female (Fig. 10). Tergite 1 with two lobes posteriorly which bear groups of six to seven long setae of about equal length. Tergal plate 2 shorter than in *T. blainvillii*. Posterior processes rather short, slightly turned outwards, with three to four setae which are about as long as those of tergite 1 and some short spines. Short setae on the surface, except in the anterior median part. Four to five long setae at the posterior lateral margins. Third tergal plate divided into two triangular plates, each of which bears three setae of which the middle one is longest. Anal segment narrow, wider basally, bare on the dorsum and with some short setae at the sides and some long setae and short spines posteriorly. Anterior part of the pleurae to about spiracle 6 covered with minute spines. Sternite 1 + 2 with a ctenidium of fifty-five to sixty teeth which are much shorter than in *T. blainvillii*. Postspiracular plate broadly rounded with only five to six minute spines at the posterior margin. Lateral sclerites of sternite 5 narrow and long, reaching closer to the median line than in *T. blainvillii* and only two setae between them. Plates of sternite 6 about twice as wide. The lateral setae are not much longer than the median ones. Sternite 7 broadly rounded, with a row of long setae posteriorly which are more widely spaced laterally and a group of closely standing setae in the middle which become shorter medially. Anal sclerite small and elliptical with two longer and two shorter setae. Adanal plates with about four longer setae and two or three short spines. Genital plate small, rounded, with minute hairs and three longer setae posteriorly and two shorter ones in the middle (Fig. 11).

Abdomen male. Tergite 1 with straight posterior margin which bears a row of moderately long setae with a short gap in the middle. Tergites 2 to 6 narrow with marginal rows of moderately long setae and some shorter ones. These setae are



Figs. 9–13. *Tripselia brevipes* n.sp. 9, posterior margin of sternal plate of thorax. 10, female, abdomen, dorsal. 11, female, genital plate and anal sclerites. 12, male, sternite 5 and genital area. 13, male: (*a*) genitalia, (*b*) aedeagus and paramere of subspecies from Sumatra.

longer on the posterior tergites and tergite 5 and 6 bear in addition two to four much longer setae near the middle. The surface of the tergites bears groups of short setae which become less numerous on the posterior tergites so that there is only a single row on tergite 6 or it may be bare. Anal segment narrow, nearly parallel sided, about as long as the three preceding tergites, with short setae in

the posterior part of the dorsum, two long setae in the middle and some long setae and short spines posteriorly. Sternite 1 + 2 as in the female. Postspiracular plate sometimes with a longer seta towards the spiracle. Sternite 5 (Fig. 12) short, not much longer than sternite 3. A double row of short spines in the middle of the posterior margin. Twelve to fourteen longer and more widely spaced spines in the posterior row and about the same number of more closely standing, shorter spines in the anterior row. A row of very long, long and shorter setae at the sides of the posterior margin.

Genitalia (Fig. 13). Basal arc rounded, with moderately long anterior process. Claspers similar as in *T. blainvillii*. Phallobase concave dorsally with a transverse row of four setae near the base and another pair distally. Aedeagus with a more or less sharp angle ventrally near the base, widening slightly in the apical half and then tapering to a blunt point. Apodeme short with a wide endplate. Parameres with blunt tip and obtuse ventral angle which sometimes bears a short tooth.

Material examined. Boentok, *Central Borneo*, Baribok. (5 ♂, 5 ♀) *Pipistrellus imbricatus*. (Shortridge.) Astoli, Bolgaum, Bombay. (1 ♂) *Tylonycteris pachypus*. (Wroughton.)

(5) **Tripselia brevipes** (?) subsp.

The two specimens from Tarussan Bay, Sumatra, mentioned by Scott, resemble *T. brevipes* closely. The anal sclerite of the female has only two setae and there are only three short setae on the adanal plates. The setae at the posterior margin of sternite 7 are shorter and the 3rd tergal plate seems undivided. In the male there are sixteen spines in the posterior row of sternite 5 and twelve in the anterior row. There are no very long setae in the marginal rows of tergite 5 and 6. The parameres are broadly rounded anteriorly and ventrally (Fig. 13*b*). If the above differences should prove constant, this form will have to be considered as a separate subspecies.

(6) **Tripselia hirsuta** n.sp.

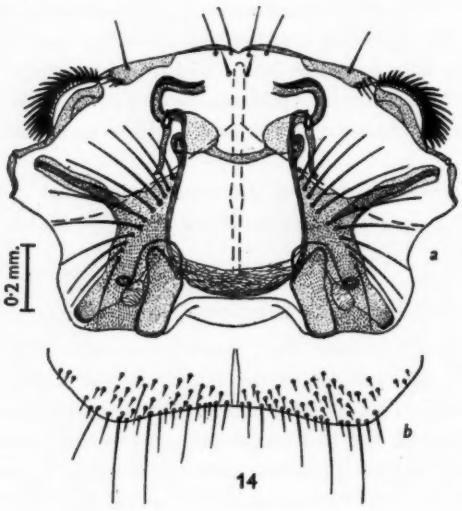
This species is of particular interest as its habitus is so very different from the type species. In its broad build and dense cover of setae it resembles much more a *Penicillidia* than a *Tripselia*. The structure of the abdomen and of the genitalia in both sexes, as well as other characters, clearly prove, however, that it belongs to the genus *Tripselia*.

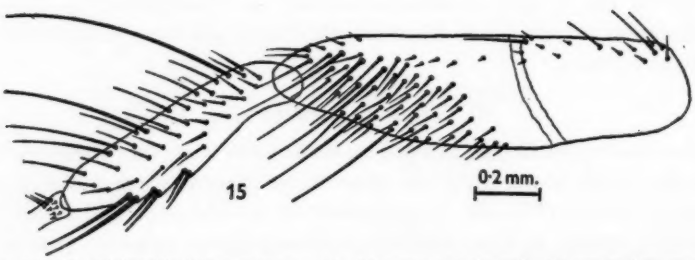
Description. Length 2·5 mm. Colour light brown.

Head laterally compressed. Anterior dorsal margin forming an angle with the apex posteriorly. At each side of the angle a row of five to six setae. Eight to ten setae on the genae. Labella of the labium as long as the theca or slightly shorter.

Thorax (Fig. 14). Much wider than long. Length to width = 2 : 3·5. Oblique sutures forming an angle of about 100°. Posterior margin of the ventral plate with a row of eight long setae, of which the lateral is longest, and shorter setae of different length between the long ones. Mesonotum wide, rounded posteriorly.

Lateral plates of the notopleural sutures wide, with a row of about twelve notopleural setae which reach nearly to the anterior spiracle. Legs of moderate length (Fig. 15). Tibiae scalpel-shaped, about four times as long as wide, with three rows of rather short setae in the distal half of the ventral surface. The setae of the distal row are longest, reaching slightly beyond the tip of the tibia and those of the proximal row just reach the base of the middle row. Anterior surface of femur 1 covered with short setae and some long ones distally. On femur 2 a bare patch near the base. The anterior surface of femur 3 has long and short setae only in the distal half.

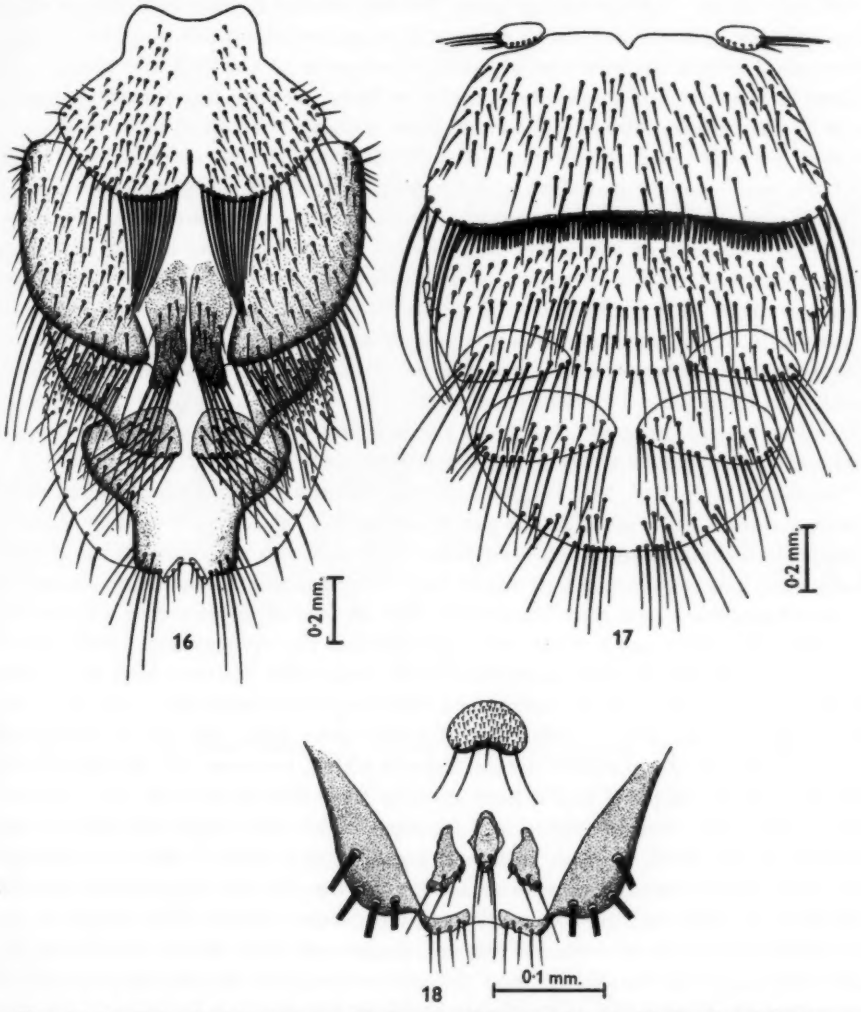




Figs. 14, 15. *Tripselia hirsuta* n.sp. 14, thorax: (*a*) dorsal, (*b*) posterior margin of sternal plate. 15, hind leg, anterior surface.

Abdomen female (Figs. 16–18). Tergite 1 rounded posteriorly with two lobes, each of which bears a dense row of about fifteen long setae. A gap in the middle between the rows. The setae become gradually shorter laterally. The surface of the tergite is covered with short setae which leave a median stripe bare. Tergal plate 2 divided into four longitudinal, sclerotized stripes. The two median ones are narrow and pigmented and bear four to six long setae at the posterior processes and a dense group of short setae anterior to them, leaving a bare space beneath the long setae of tergite 1. The two lateral areas are wide, densely

covered with short setae and bear a row of about twelve long setae at the posterior margin. Tergal plate 3 divided into two rounded plates covered with setae of moderate length and with some long setae posteriorly. On the connective membrane behind the lateral areas of tergal plate 2 a dense group of moderately long



Figs. 16–18. *Tripselia hirsuta* n.sp. 16, female, abdomen, dorsal. 17, female, abdomen, ventral. 18, female, genital plate and anal sclerites.

setae. Pleurae covered with minute spines anteriorly to about spiracle 6 and bare farther posteriorly. An oblique row of setae between spiracles 6 and 7. Anal segment narrow posteriorly, with two lateral bulges anteriorly, bare dorsally. Some longer and shorter setae on the anterior lateral bulges and some moderately long setae in the posterior lateral corners. Sternite 1 + 2 wide, trapezoid, with a

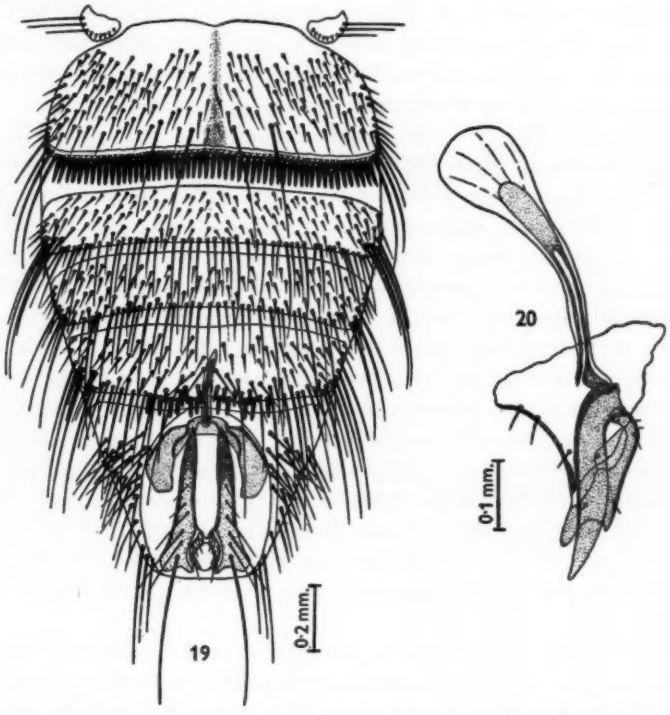
ctenidium of about seventy teeth which are longer laterally and three to five very long curved setae at the sides of the ctenidium in the posterior lateral corners. The surface of the sternite is covered with short setae except for a bare anterior and posterior stripe. Behind the field of short setae a row of eight to ten long setae. Postspiracular plate broadly rounded with two longer setae towards the spiracle and six to seven minute spines along the posterior margin. Sternite 3 with a uniform row of rather short setae at the posterior margin and three to four rows of short setae on the surface. Six long setae in the posterior row of the surface. Sternite 4 narrow, bare, with a row of short setae posteriorly which does not reach to the sides. Sternite 5 with a similar marginal row in the middle and two narrowly triangular sclerites laterally. These sclerites bear a double row of long setae posteriorly which stand more densely at the sides. Sternite 6 consists of two larger elliptical sclerites with longer and shorter setae posteriorly and one or two rows of short setae on the surface. Sternite 7 very wide, so that it is visible dorsally at the sides of the anal segment, rounded posteriorly. It bears longer setae posteriorly and several rows of shorter setae in the lateral posterior corners. Anal sclerite small, narrow, elliptical or rhomboid, with two longer and two shorter setae posteriorly. Adanal plates with three to four longer and some short setae. Genital plate transversely elliptical, with minute hairs and four to six longer setae posteriorly (Fig. 18).

Abdomen male (Fig. 19). Tergite 1 short, with straight posterior margin which bears a row of short setae with a gap in the middle. Three rows of short setae in the posterior lateral parts of the surface. Tergites 2 to 6 with rows of moderately long setae posteriorly and four to six very long and thick setae in the marginal rows of tergites 4 to 6 near the middle. The surface of tergites 2 to 5 is densely covered with short setae which are longer than in the other species. Only one to two rows on tergite 6. Anal segment broadly triangular, covered with short setae dorsally in the posterior two-thirds and with long setae posteriorly. Sternite 1+2 as in the female, with a ctenidium of about sixty teeth and one to three long curved setae at the posterior lateral corners which, however, are shorter than in the female. Sternites 3 and 4 narrow, with three to four rows of short setae on the surface and marginal rows of long setae which are longer and stand more densely at the sides. Sternite 5 slightly longer than sternite 4 with long setae at the sides of the posterior margin and a double row of short spines in the middle. There is a small indentation in the middle of the margin. The spines of the posterior row are about twelve in number, longer and more widely spaced laterally and with a gap in the middle, near the indentation. The anterior row consists of about twelve shorter and more closely standing spines.

Genitalia (Fig. 20). The sides of the ventral surface of the anal segment are densely covered with moderately long setae. Claspers rather short, slightly curved, pigmented, with a long seta dorsally near the base and a dorsal row of shorter setae. Basal arc broad, with wide lateral flaps and long anterior process. Phallobase slightly concave dorsally with two pairs of setae near the base and another pair farther distally. Aedeagus slightly curved, wide in the basal two-thirds and then tapering to a blunt tip. Apodeme short with a wide end-plate.

Parameres with long distal process with a rounded tip and a sharp ventral tooth. A deep concavity between the tip and the ventral tooth.

Material examined. *New Guinea*, Kamali, on *Nycticeius greyi* (4 ♀, 2 ♂) and on *Pipistrellus javanicus abramus* (5 ♀, 2 ♂). Types and paratypes in the British Museum (Nat. Hist.).



Figs. 19, 20. *Tripselia hirsuta* n.sp. 19, male, abdomen, ventral. 20, male genitalia.

(7) Tripselia falcozi Musgrave, 1925

Syn. (?) *Nycteribia oceanica* Speiser, 1901 (nec Bigot, 1885).

Musgrave (1925, 1927) described a number of species of *Nycteribia* which apparently belong to the genus *Tripselia*. Of these only two paratypes of *T. falcozi* were available for examination and some unidentified material. Musgrave's species will have to be re-examined and the genitalia described. It does not seem advisable to describe the several apparently new species as they may prove to belong to species already described by Musgrave. *Tripselia falcozi*, however, is re-described in order to complete Musgrave's description with some essential details, mainly of the structure of the genitalia. Musgrave (1927) noted the resemblance of his *Nycteribia falcozi* to *Tripselia blainvillii*, but did not place it in this genus because of differences which have now proved to be of specific importance only. As will be seen from the following description and the figures, *T. falcozi* resembles *T. blainvillii* closely, except for differences mainly in the length of the legs and the genitalia.

Description. Length 2·2 to 2·5 mm. Colour light brown. *Head* laterally compressed with six setae at the anterior dorsal margin. Labella of the labium slightly shorter than the theca.

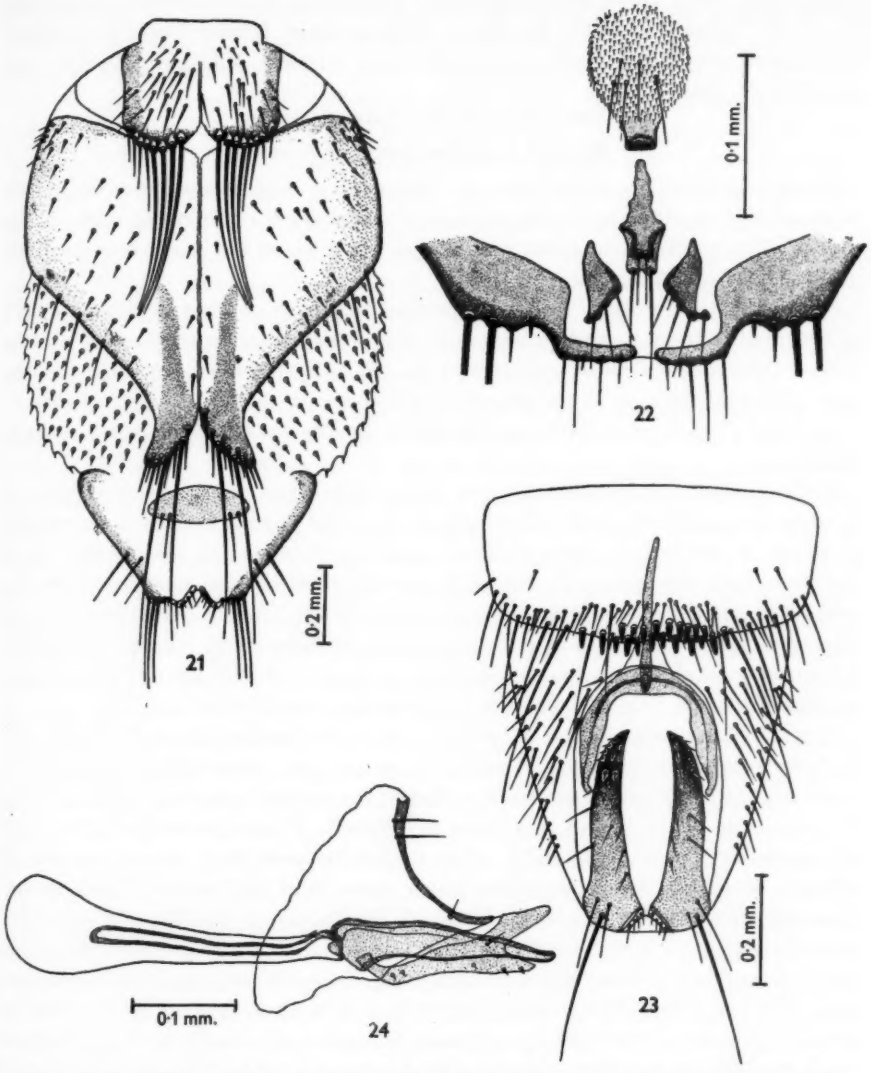
Thorax as in *T. blainvillii*, slightly wider than long. A row of 6 long setae and some shorter setae at the posterior ventral margin. The lateral setae nearly as long as sternite 1 + 2. Legs moderately long, tibiae slender, 5 to 6 times as long as wide. Three rows of long setae at about the middle of the ventral surface of the tibia, the long setae of the distal row reaching the tip of the tibia, those of the proximal row short, reaching the base of the middle row.

Abdomen female (Figs. 21, 22). Tergite 1 similar as in *T. blainvillii* with two broad posterior lobes, which bear four to five long setae and four to six short setae laterally. Tergal plate 2 heart-shaped with two pointed posterior processes which bear two to three long setae and a row of six to seven short spines. The long setae are slightly longer than those of tergite 1. Two pigmented stripes as in *T. blainvillii* from the posterior processes into the middle of the lateral areas. Four moderately long setae at the lateral posterior margins. Short setae at the sides of the surface and in a single row along each side of the median line. Tergal plate 3 transversely elliptical, with two longer and two short setae. Anal segment broadly triangular, bare dorsally, with two to three short setae laterally and posteriorly and two to three long setae and some short spines at the posterior end. Ventral surface as in *T. blainvillii*. Postspiracular plate broad, with two to three long setae towards the spiracle and six to seven minute spines along the posterior margin. Anal sclerite parallel sided, with two longer and two shorter setae posteriorly. Adanal plates as in *T. blainvillii*. Genital plate pear-shaped, covered with minute hairs and with a double row of five to six setae in the middle.

Abdomen male (Fig. 23). Similar in general to that of *T. blainvillii*. The marginal rows of the tergites consist of longer setae alternating with short spines. The whole surface of tergite 2 is covered with short setae, tergite 3 only in the middle. Only a single row on the surface of tergite 4 and 5 and tergite 6 bare. Sternite 5 broad, with a double row of short spines in the middle of the posterior margin. There are twelve more widely spaced spines in the posterior row and nine more closely standing spines in the anterior row. Some long setae at the posterior margin lateral to the rows of spines and a row of setae anterior to them which are shorter in the middle and longer laterally.

Genitalia (Fig. 24). Claspers rather thick, slightly curved, with the usual long seta dorsally near the base and a few rather short setae dorsally. Basal arc rounded, with a very long anterior process. Phallobase strongly concave dorsally, with two pairs of setae near the base and another pair apically. Aedeagus nearly straight, tapering to a blunt point. Apodeme short with moderately wide end-plate. Parameres with long distal process and a long ventral tooth. A deep concavity between the distal process and the ventral tooth. Some minute hairs along the ventral margin and a longer hair near the base of the distal process.

Two specimens from Alexandria, North Australia, and a male from N. Queensland resemble *T. falcozi* closely, but differ in minor details in the ♂ and ♀ genitalia.



Figs. 21–24. *Tripselia falcozi*. 21, female, abdomen, dorsal. 22, female, genital plate and anal sclerites. 23, male, sternite 5 and genital area. 24, male, genitalia.

The aedeagus is slightly curved, the ventral tooth of the paramere is shorter and there are only six spines in the anterior row of spines on sternite 5. The anal sclerite in the female is of slightly different shape. Whether these differences are significant will have to be determined by examination of more material.

Material examined: Mt Lyndhurst, *South Australia*, Farina. (1 ♂, 1 ♀) (Paratypes) *Chalinolobus gouldi*. (Troughton.) *N.S. Wales*, Paramatta. (1 ♂, 1 ♀)

Chalinolobus moris. (Rothschild collection.) Alexandria, *North Territory, Australia*. (1 ♂, 1 ♀) *Chalinolobus gouldi*. (Stalker.) *N. Queensland*, Dinner Creek, Ravenshoe. (1 ♂) *Eptesicus pumilus*. (Rothschild collection.) *Victoria*, Mildura. (1 ♀) *Eptesicus pumilus*. (Mackerras.)

Genus **Basilia** Mir. Ribeiro, 1903

(8) **Basilia bathybothyra** Speiser, 1907

Speiser's preliminary description was based on a single male found on *Myotis muricola* in Calcutta and is not sufficiently detailed for the recognition of the species. This specimen was lost during the last war, as Dr A. P. Kapur of the Indian Museum informs me.

Scott (1914) described *Penicillidia fletcheri* var. *pumila* from Ceylon. In 1917 he recorded it from Khandala, Bombay, but re-examination of these specimens proved them to be *Basilia majuscula* n.sp. In 1925, after having examined the type of *B. bathybothyra*, he considered *P. fletcheri* var. *pumila* as identical with it.

In 1954 Theodor and Moscona described *Basilia bathybothyra daganiae* from Palestine.

In the present paper several forms from India and Africa are described, all of which have hitherto been considered as *B. bathybothyra*. It is thus not certain to which of the Indian forms Speiser's specimen belonged as the genitalia were not described. The name *B. bathybothyra* will therefore be considered for the present to apply to Speiser's specimen only and the various forms will be considered as species as all show clear and constant differences. One of the Indian forms will probably prove to be identical with *B. bathybothyra* of Speiser when material from the Calcutta region is available for examination.

Scott (1925, 1936) referred *B. pumila*, which he considered as identical with *B. bathybothyra*, to the genus *Penicillidia*, as the eyes apparently consisted of a single ocellus. This fact alone is not sufficient for placing a species in *Penicillidia*. The structure of the eye in this genus is different. There is a half spherical, unpigmented ocellus in *Penicillidia*, while in *Basilia* there is a pigmented base of different structure which protrudes much more from the surface (Theodor and Moscona, 1954, p. 202, fig. 52). Although specimens of *Basilia* as a rule have eyes consisting of two (sometimes three) ocelli, there are specimens in which only one ocellus is present on one or both sides. Or, as the eye consists of a common base with two incompletely divided ocelli, one of them is covered by the other in certain positions so that the eye appears to consist of a single ocellus. Furthermore, the differences between the genera *Basilia* and *Penicillidia* are well marked in other respects (structure of the thorax, presence of haltere covers and absence of the postspiracular plate in *Penicillidia*, genitalia, etc.) so that there is no doubt that the various forms of the *Basilia bathybothyra* group belong to the genus *Basilia*.

(9) **Basilia pumila** Scott, 1914

Syn. *Penicillidia fletcheri* var. *pumila* Scott, 1914.

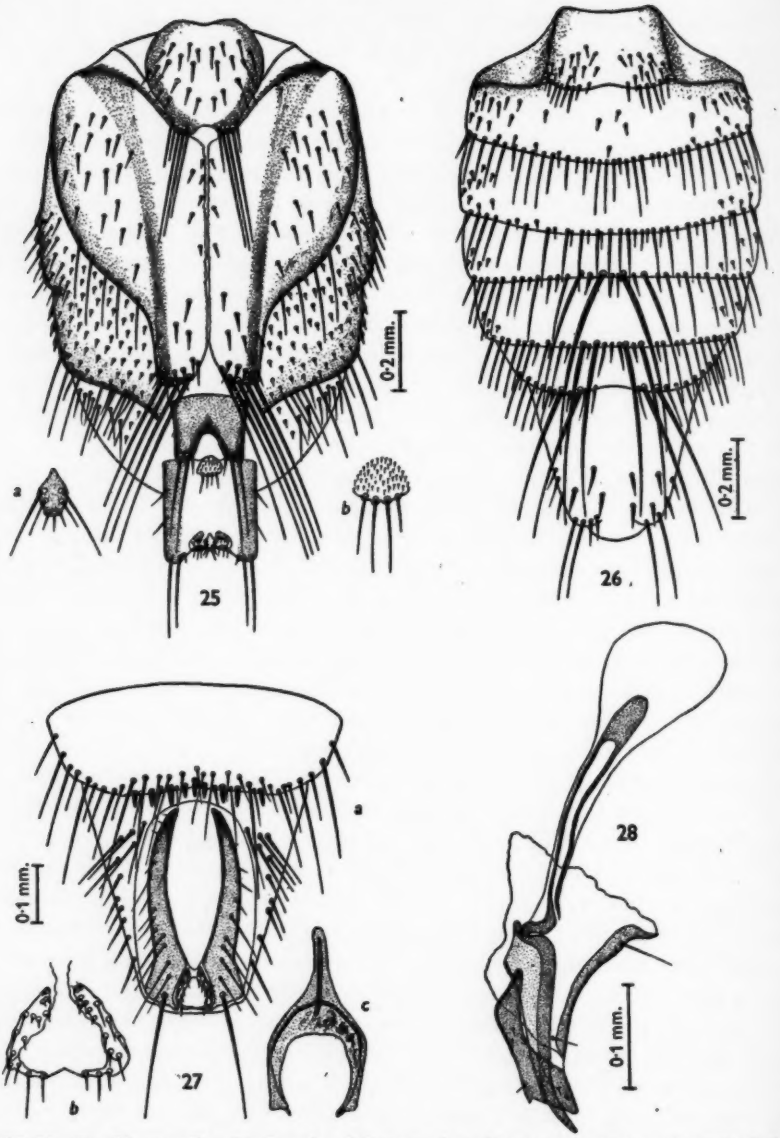
Description. Length 1·5 mm. Colour yellowish.

Head laterally compressed with straight anterior dorsal margin which bears four setae. Two more between the eyes. These are pigmented, nearly spherical

and there is no indentation between the ocelli. Sometimes only a single ocellus on one or both sides. Labella of the labium rather thick, about half as long as the theca or slightly less.

Thorax wider than long. Length to width = 4 : 5. Median sternal suture indistinct in the middle third. The oblique sutures form an angle of about 95°. The small hairs of the ventral surface are very short anteriorly and longer posteriorly. The posterior margin of the sternal plate bears six long setae, of which the lateral are longest, and between them short setae. Mesonotum wide. Lateral plates of the notopleural sutures wide with six to nine notopleural setae. Mesopleural sutures distinct, narrow. Thoracic ctenidia with about twenty pointed spines. Legs rather short, tibiae scalpel-shaped, four times as long as wide, with three rows of setae in the distal half of the ventral surface. The setae of the distal row are longest and extend beyond the tip of the tibia.

Abdomen female (Fig. 25). Tergite 1 with two lobes posteriorly which bear three to four moderately long, thin setae and one or two shorter ones. Short setae on the surface which are longer and thicker laterally. Tergal plate 2 heart-shaped, divided in the middle, with broad posterior processes which bear four to five very long and thick setae. These are nearly twice as long and thick as those of tergite 1. Near them a row of five to seven short spines. Four to five moderately long setae along the posterior lateral margins. Short setae in the lateral parts of the surface and a few along the median line. A transverse row of stronger spines at the base of the posterior processes. A narrow pigmented stripe runs from the outer side of the posterior processes through the middle of each half of the tergal plate to the shoulders. Tergal plate 3 rather long with two posterior processes which bear two thick, moderately long setae and a short spine. Pleurae covered with minute spines. Anal segment short, parallel sided, with two or three short setae laterally and two long setae and some short spines at the posterior processes. Sternite 1 + 2 wide and long, rounded anteriorly, with a ctenidium of about sixty teeth. Surface covered with short setae in the posterior two-thirds. Postspiracular plate wide, with two to three long setae towards the spiracle and five to six minute spines along the posterior edge. Sternite 3 with a uniform row of rather short setae along the posterior margin and three to four rows of short setae on the surface. Four longer setae in the posterior row of the surface. Sternite 4 concave posteriorly with a similar marginal row which, however, reaches farther laterally. Only a single row of short spines on the surface which may be broadly interrupted in the middle and two to three rows laterally. Sternite 5 with two narrowly elliptical sclerites laterally which do not quite reach the median line. They bear a single row of moderately long setae towards the middle and two or three rows laterally. One or two of the lateral setae are much longer than the others. Sternite 6 with two broader rectangular sclerites which reach the median line, with similar marginal rows and a row of short setae on the surface. Sternite 7 trapezoid or rounded posteriorly with a row of long setae posteriorly with a gap in the middle and two transverse rows of short setae in the posterior half. Two long and thin setae in the posterior row of the surface. Anal sclerite small, rhomboid or drop-shaped with two longer setae anteriorly and



Figs. 25–28. *Basilia pumila*. 25, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 26, male, abdomen, dorsal. 27, male: (*a*) sternite 5 and genital area, (*b*) anal frame, (*c*) basal arc. 28, male, genitalia.

four to five short ones posteriorly. Adanal plates not clearly differentiated. Genital plate small, rounded, with four to six setae posteriorly. The field of minute hairs is not so clearly marked as in *Tripselia*.

Abdomen male (Figs. 26, 27). Tergite 1 with straight posterior margin which bears a row of short setae with a gap in the middle. Tergite 2 wide with a row of

rather short setae which are equally spaced and alternate with short spines. Some short setae in the lateral parts of the surface and a few in the middle. Tergites 3 to 6 narrow with similar marginal rows and with four very long and thick setae near the middle of the rows on tergite 4 to 6. Surface bare except for some minute hairs in the lateral posterior corners of the surface which become gradually less on the posterior tergites. A wide gap in the middle of the marginal row of tergite 6. Anal segment short, narrowly triangular with two or three long setae posteriorly and some short setae in the posterior part of the dorsum. Sternite 1+2 as in the female. Sternite 3 and 4 narrow with marginal rows of moderately long setae and three to four rows of short setae on the surface of sternite 3 and a single row on sternite 4. Two very long and thin setae laterally in this row. Sternite 5 not much longer than sternite 4, with slightly curved posterior margin. A row of eight to nine short, thick spines in the middle of the posterior margin and a row of setae which are shorter between the spines and longer laterally. Anterior to the row of spines a row of some long and short setae.

Genitalia (Fig. 28). Claspers thin, slightly curved, with pigmented tips. A long seta dorsally near the base and a row of shorter setae on the dorsal surface. Basal arc rounded, narrow, with moderately long anterior process. Aedeagus broad basally, tapering to a long point, slightly curved. Apodeme short with moderately wide end-plate. Parameres with short distal point, an obtuse ventral angle which sometimes carries a short tooth. A row of three to four minute hairs near the dorsal edge. Phallobase concave dorsally with two setae near the base and two more farther distally.

Distribution. Ceylon, Helwak, near Bombay and Madras, India. Scott's record from Khandala, Bombay, refers to *B. majuscula*.

Material examined, Ceylon. Peradeniya. *Pipistrellus coromandra.* (1 ♂, 1 ♀.) (Paratypes of *P. fletcheri* var. *pumila*.) Kakirawa, N.C.P. *P. coromandra.* (2 ♂) (Phillips). Anasigalla, Kalutara, Matugama. *P. mimus mimus.* (4 ♂, 4 ♀) (Phillips). Helwak, *India*, Bombay. (1 ♀) *P. abramus* (?). (Rothschild collection.) Madras. *P. abramus* (?). (1 ♀) (Jerdon).

(10) **Basilia majuscula** Edwards, 1919

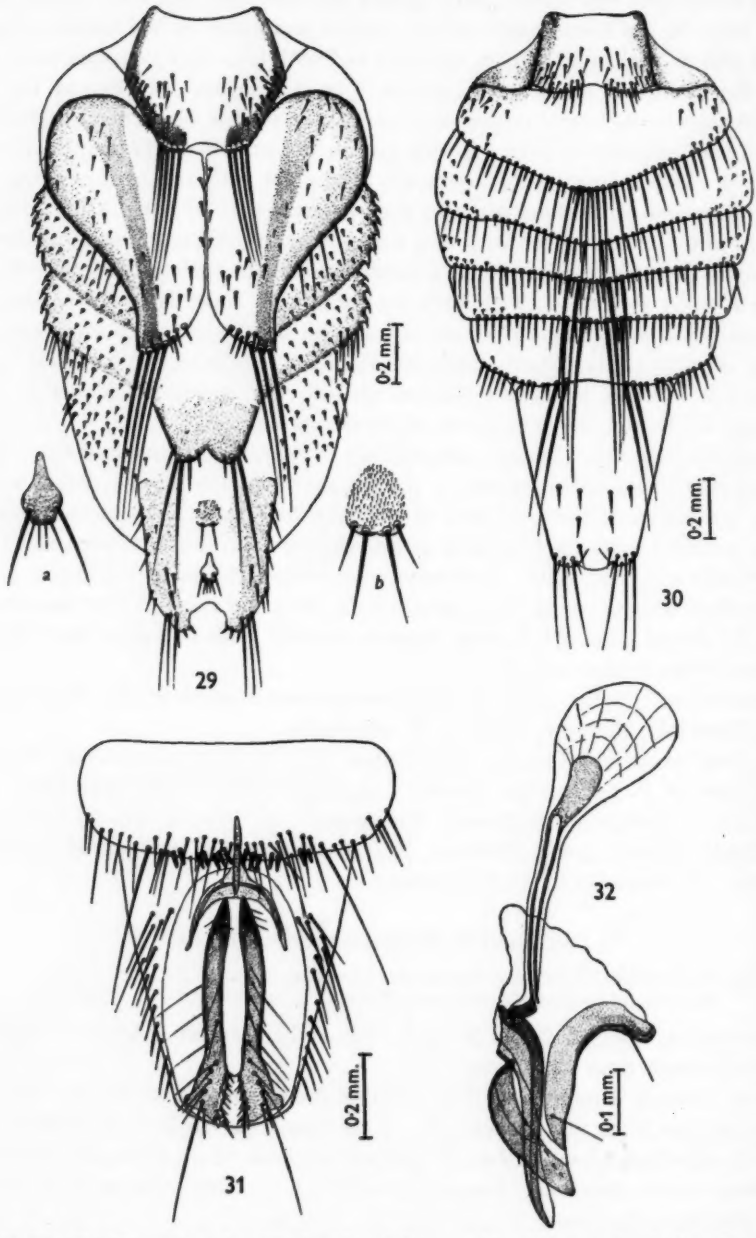
Syn. *Penicillidia fletcheri* var. *majuscula*, Edwards, 1919.
Penicillidia longiseta Schuurmans Stekhoven, 1939.

Description. Length 2·2 to 2·5 mm. Colour light brown. A much larger and slenderer insect than *B. pumila*.

Head laterally compressed. The anterior dorsal margin forms an angle with three or four setae along each side. Eyes with two small, well-marked ocelli. Labella of labium about half the length of the theca which is broadly triangular.

Thorax wider than long. Length to width = 5 : 6. Otherwise as in *B. pumila*. Legs also as in *B. pumila*.

Abdomen female (Fig. 29). The posterior lobes of tergite 1 bear three to six long and thick setae. Between the two groups a shallow concavity. Surface covered with short setae laterally. Tergal plate 2 rather short, heart-shaped, with broad, rounded posterior processes which bear three very long and thick setae, not much



Figs. 29–32. *Basilia majuscula*. 29, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 30, male, abdomen, dorsal. 31, male, sternite 5 and genital area. 32, male, genitalia.

longer than those of tergite 1 and a row of six to seven short spines. Four to five short setae at the posterior lateral margins. The pigmented stripes are broader than in *B. pumila*. The short setae on the surface distributed as in *B. pumila* but there are about eight strong spines in two to three rows at the base of the posterior processes and only a few small spines along the median line. Tergal plate 3 with shorter and broader posterior processes than in *B. pumila* which as a rule bear two longer setae and two to three short spines. Pleurae covered with minute spines. Anal segment long, nearly parallel-sided, with rather long anal processes, bare dorsally except for a few short setae in the lateral posterior corners, some similar setae along the whole length of the sides, some long setae at the base of the anal processes and a few short spines at the tip. Ventral surface similar as in *B. pumila*. Abdominal ctenidium with sixty-five to seventy-five teeth. Post-spiracular plate with three to four long setae towards the spiracle and five to six minute spines. The lateral sclerites of sternite 5 with a marginal row of longer setae and a row of short setae on the surface. Those of sternite 6 with two to three rows of short setae on the surface in addition to the marginal row. Anal sclerite pear- or drop-shaped with long anterior process, two longer setae in its broadest part and four to five short setae posteriorly. Genital plate rounded, with minute hairs and three to five setae posteriorly.

Abdomen male (Figs. 30, 31). Tergite 1 as in *B. pumila*, but the marginal setae are longer towards the middle. Tergite 2 triangularly produced in the middle of the posterior margin. At the apex stand six to eight longer setae close together. More laterally the setae are shorter, more widely spaced and alternate with short spines. Surface bare except for a group of short spines in the lateral posterior corners. Tergites 3 to 6 narrow, with marginal rows of moderately long setae alternating with short spines in the middle half and two to four very long and thick setae near the middle of tergites 4 to 6. A wide gap in the middle of the marginal row of tergite 6. Surface of tergites 3 to 6 bare except for groups of eight to twelve minute spines in the lateral corners of tergites 3 to 5. Only one or two on tergite 6. Anal segment long, narrowly triangular with six to eight short spines in the posterior part of the dorsum. Three to four long setae and some short spines posteriorly. Ventral surface as in *B. pumila*. Sternite 5 with an irregular row of nine to twelve short spines in the middle of the hindmargin.

Genitalia (Fig. 32). Claspers long and thin, pigmented, with setae as in *B. pumila*. Basal arc narrow, rounded, with long anterior process. Phallobase strongly concave dorsally with two setae near the base and two more distally. Aedeagus long, slightly curved, tapering to a point. Apodeme with wide end-plate. Parameres long, narrow, curved ventrally with short and blunt distal end and a row of about six minute hairs along the dorsal edge.

Distribution. Java, Sumatra, India.

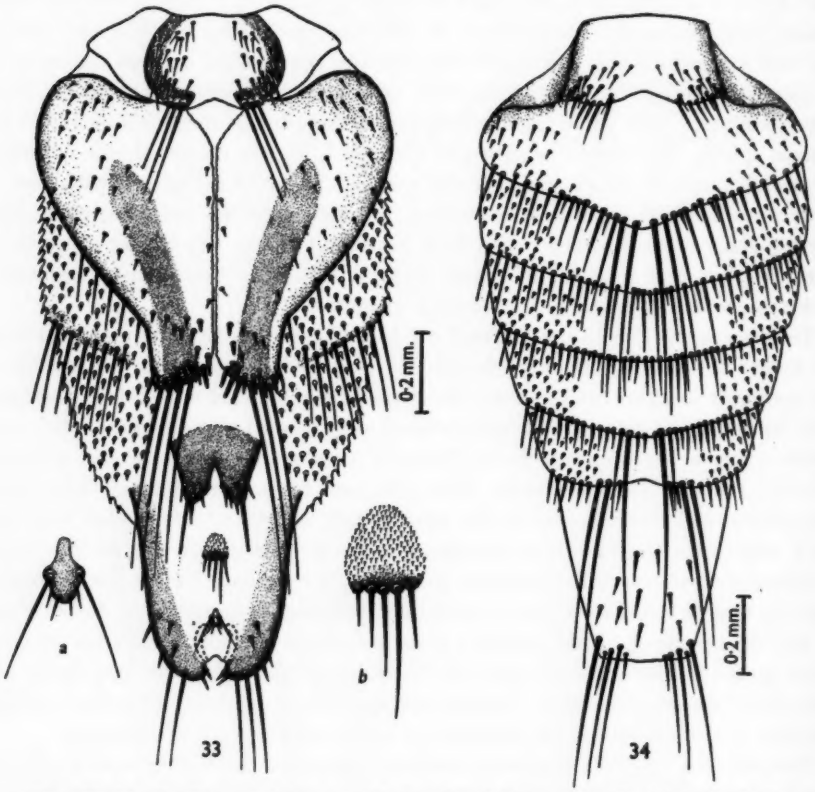
Material examined. *West Sumatra*, Mt. Korinchi, on *Vespertilio* sp. (2 ♂, 2 ♀) Cotypes. (Robinson & Kloss.) *Java*, Buitenzorg, *Tylonycteris pachypus*. (3 ♀) (Balston & Shortridge). West Java, *Pipistrellus tralatitius*. (2 ♂, 6 ♀) (Shortridge). Khandala, Bombay. (1 ♀) (Assmuth). 'India' (1 ♀). Two females in the Vienna Museum designated by Speiser as *Basilia tetracyrta* without locality and host also belong to this species.

(11) **Basilia punctata** n.sp.

This species resembles *B. majuscula* closely, but differs in a number of chaetotactic details.

Description. Habitus, length, colour, head and thorax as in *B. majuscula*.

Abdomen female (Fig. 33). The setae at the posterior processes of tergite 1 are much shorter and thinner and the gap between them is wider than in *B. majuscula*. They are about one-third of the length of the long setae of the posterior processes of



Figs. 33, 34. *Basilia punctata* n.sp. 33, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 34, male, abdomen, dorsal.

tergal plate 2. The posterior processes of tergal plate 2 are broader than in *B. majuscula*. They bear three to five very long and thick setae and a row of seven to ten short spines. The setae of the surface are shorter than in *B. majuscula*. The pigmented stripes are broader and end in the middle of the tergal plate. Tergal plate 3 as in *B. majuscula*, but the posterior processes are longer and bear only one moderately long seta and four to six longer and shorter spines. Anal segment bare dorsally and at the sides except for a few spines in the lateral posterior corners, two to three long setae and some spines at the anal processes

which are rather long and curved towards the middle. Anal sclerite and genital plate as in *B. majuscula*. Ventral surface as in *B. majuscula*. Post-spiracular plate narrow, curved, with three long setae towards the spiracle and three to four minute spines at the posterior margin.

Abdomen male (Fig. 34). Tergite 2 even more triangularly produced in the middle of the hindmargin than in *B. majuscula*. The group of long setae in the middle of the marginal row consists of four to six very long and thick setae, which are much longer than in *B. majuscula*. Tergites 3 to 6 with marginal rows of rather short setae, most of them about half as long as the tergites, and some short spines between them. There are two very long setae near the middle of the marginal row of tergite 4 which are absent in one specimen, two on tergite 5 and four on tergite 6. The surface of tergites 3 to 6 is covered with minute thick spines in their lateral thirds or quarters, about forty to fifty in number as against ten to twelve in *B. majuscula*. Ventral surface and genitalia as in *B. majuscula*. The basal arc seems wider and the row of spines at the posterior margin of sternite 5 consists of fifteen spines in an irregular double row in one specimen, of a single row of eleven in another and of nine in a third.

Distribution. Ceylon, South India.

Material examined. *Ceylon*. Namunokula, Uva Prov. *Pipistrellus ceylonicus*. (3 ♀, 1 ♂) (Phillips). Ohyia. *P. ceylonicus*. (1 ♂) (Phillips). *India*, Bangalore, Mysore, (1 ♂) (Assmuth).

B. punctata resembles *B. majuscula* so closely, particularly in the structure of the genitalia, that it may be justifiable to consider it as a subspecies of *B. majuscula*. There is so little information, however, about the distribution of both species and the variation of characters that this question is left open for the present.

(12) **Basilia fletcheri** Scott, 1914

Syn. *Penicillidia fletcheri* Scott, 1914.

The species has been described in detail by Scott and the present description is intended mainly to bring it into line with descriptions of related species and to add details not given by Scott. There is no doubt that the species belongs to the genus *Basilia* in all essential characters. The eyes are of *Basilia* type although the ocelli are difficult to distinguish in the few specimens available.

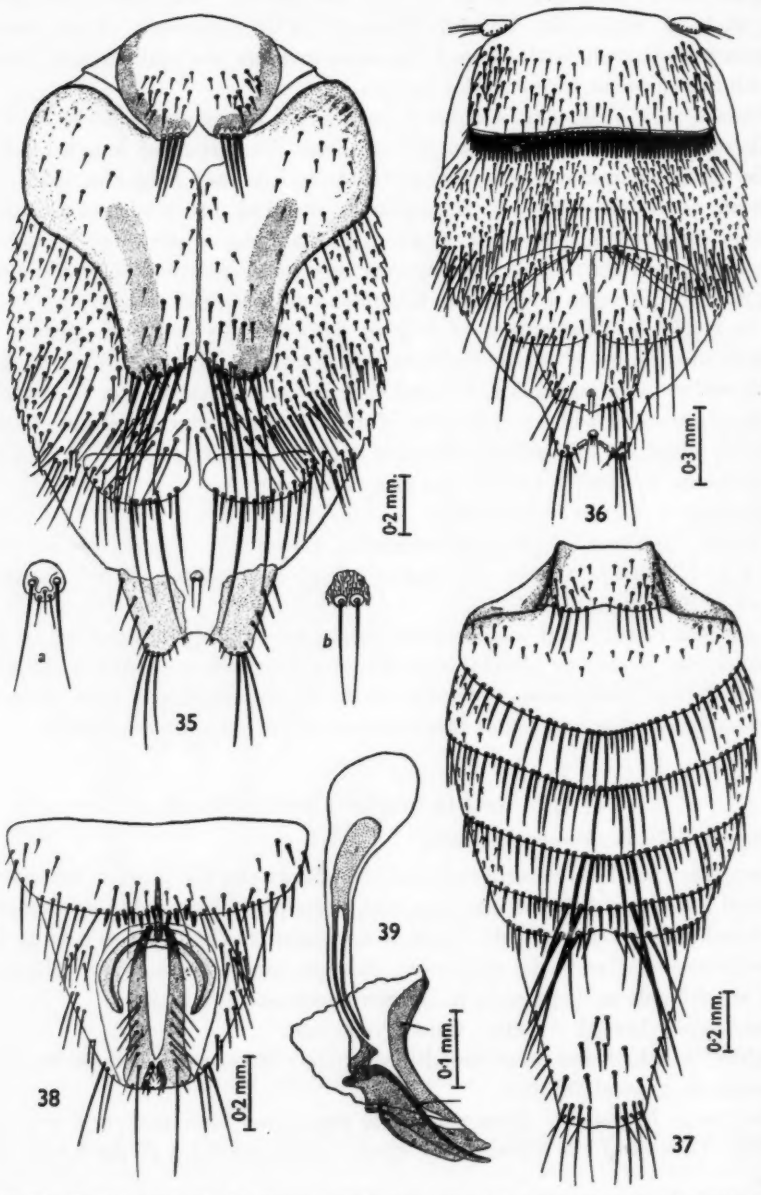
Description. Length 2·5 mm. Colour yellowish.

Lighter in colour and more broadly built than *B. majuscula*, more resembling *B. pumila* in general habitus.

Head as in *B. pumila*. Eyes with wide pigmented base and ocelli not clearly divided. Labella of the labium very short, about one-third of the length of the theca.

Thorax and legs as in *B. pumila*.

Abdomen female (Figs. 35, 36). Tergite 1 rounded posteriorly with two groups of four short and thick setae posteriorly and an indentation between them. Tergal plate 2 short, heart-shaped, with very broad posterior processes which bear five to six long, thick setae which are shorter than in *B. majuscula*. They are about



Figs. 35–39. *Basilia fletcheri*. 35, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 36, female, abdomen, ventral. 37, male, abdomen, dorsal. 38, male, sternite 5 and genital area. 39, male, genitalia.

three times as long as those of tergite 1. A row of eight to ten short spines near the long setae. Five to six rather short setae at the posterior lateral margins. Short setae in the anterior lateral parts of the tergal plate, along the median line and longer spines near the base of the posterior processes. Tergal plate 3 divided into two large, transversely elliptical sclerites which bear about four long setae posteriorly towards the middle and shorter setae between them and towards the sides. A row of short setae laterally on the surface. The connective membrane between tergal plate 2 and 3 is densely covered with moderately long setae. The minute spines on the pleurae are longer and thinner than in the other species. Behind tergal plate 3 dorsum and pleurae are bare. Anal segment very short, bare dorsally and with several setae at the sides. Three to four long setae and some short spines at the anal processes. Sternite 1 + 2 with a ctenidium of about seventy teeth. Postspiracular plate as in *B. majuscula*. Sternite 3 concave posteriorly with a marginal row of setae which are short in the middle and longer laterally. About six rows of short setae on the surface which are longer in the middle and minute laterally. Four to six long, thin setae in the posterior row of the surface. Sternite 4 very narrow, with a similar marginal row as sternite 3, bare on the surface except for two or three long setae at the sides. Sternite 5 with two narrow sclerites which stand obliquely and do not quite reach the median line. Two setae between the sclerites, close to the anterior margin of sternite 6. The marginal rows are similar to those of the preceding sternites and there is a row of short setae on the surface of the sclerites. Sternite 6 divided into two broad, triangular sclerites which touch in the median line. A row of moderately long setae at the posterior margin of which two at the sides are much longer and three to four rows of short setae on the surface. Sternite 7 rounded posteriorly with a small process in the middle. A row of long setae at the hind margin with a gap in the middle and two to three rows of short setae in the lateral posterior corners of the surface. Anal sclerite small, rounded with two longer setae basally and two to three short setae posteriorly. Genital plate small, rounded, with minute hairs and two setae.

Abdomen male (Figs. 37, 38). Tergite 1 with short setae posteriorly with a concave gap in the middle. Tergite 2 uniformly rounded posteriorly, not produced in the middle as in *B. majuscula*. The setae are uniformly spaced and alternate with short spines. The median setae are not much longer than the lateral ones. A transverse row of short spines, which is double laterally, across the middle of the surface. Tergites 3 to 6 narrow, with marginal rows of moderately long setae alternating with short spines, bare on the surface except for some minute spines at the sides which become gradually less on the posterior tergites. Two to four very long setae in the middle of the marginal rows of tergite four to six and a gap in the marginal row of tergite 6. Sternite 4 with a double row of short spines on the surface. Sternite 5 not much longer than sternite 4, with an irregular row of nine to twelve short spines in the middle of the posterior margin.

Genitalia (Fig. 39). Claspers long and thin, slightly curved. Basal arc rounded, with long anterior process. Phallobase concave dorsally with two setae rather far from the base and two setae apically. Aedeagus broad at the base, slightly

curved, tapering to a long point. Apodeme with moderately wide end-plate. Parameres rather short with a sharp point distally and obtuse ventral angle. A row of minute hairs along the dorsal edge.

Material examined. Coimbatore, Madras. *Pipistrellus dormeri.* (1 ♂, 1 ♀.) Types. (Fletcher). Navapur, 200 m. N.N.E. of Bombay. *P. dormeri.* (1 ♀, 2 ♂) (Rothschild Collection). Bolgaum, Bombay. *Tylonycteris pachypus.* (1 ♂) (Wroughton).

The specimen from Bangalore, Mysore, referred by Scott to this species belongs to *B. punctata.*

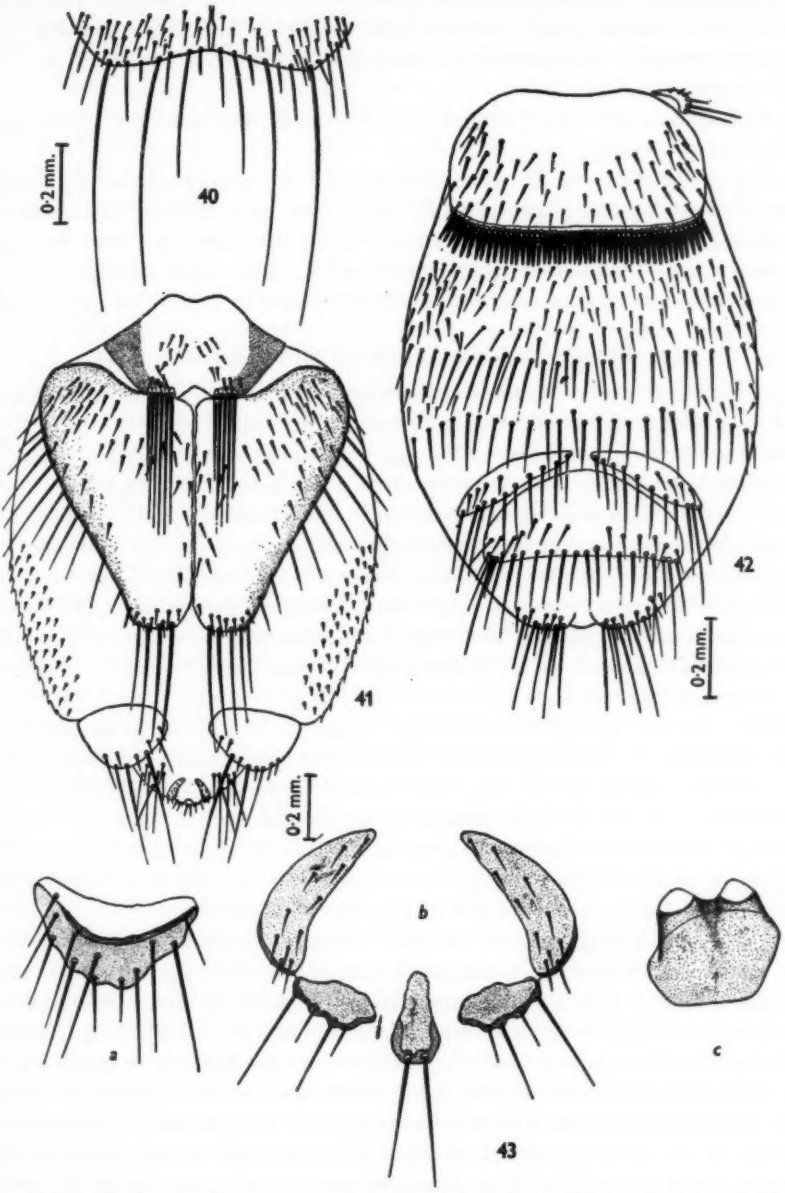
(13) **Basilia seminuda** n.sp.

Description. Length 2 mm. Colour yellowish, lightly sclerotized.

Head as in *B. pumila.* Eyes shallow with wide posterior opening of the base and two well-separated ocelli (Fig. 43*c*). Labella of the labium very short, about one-quarter of the length of the theca.

Thorax (Fig. 40). Sternal plate wider than long. Length to width = 4 : 5. Median sternal suture narrow, distinct. Oblique sutures forming an angle of 90°. Six very long setae at the hind margin of the sternal plate, the four lateral ones reaching to the hind margin of sternite 3, and between them shorter setae. Dorsal pattern of the thorax as in *B. pumila.* Eight to nine notopleural setae. Legs also as in *B. pumila.*

Abdomen female (Figs. 41–43). Tergite 1 bears two groups of five to six long setae with a wide gap between them. Tergal plate 2 heart-shaped with nearly straight lateral borders which bear a row of about ten moderately long setae. The posterior processes are broad and bear four to five setae which are about as long as those of tergite 1. Near them a row of seven to eight short spines. A dense group of short setae in the anterior lateral parts of the surface and some along the median line. Tergal plate 3 divided into two roughly triangular plates which are widely separated and lie far posteriorly. They bear three to four long setae and some short ones posteriorly and are bare on the surface. The connective membrane between tergal plate 2 and 3 and the median part of the pleurae bare. Anal segment very short, triangular, with a group of long and short setae posteriorly. Anal frame wide, with a double row of minute spines. Sternite 1+2 with a ctenidium of fifty-five long teeth. Postspiracular plate narrow, curved, with three long setae towards the spiracle and three minute spines at the posterior edge. Sternite 3 with a uniform row of moderately long setae posteriorly and about six rows of short spines on the surface which are much longer posteriorly. Four long setae in the posterior row of the surface which are as long as those of the marginal row. Sternite 4 with a similar marginal row and some short setae laterally on the surface. Sternite 5 consists of two narrowly elliptical plates which stand obliquely and do not quite reach the median line. They bear moderately long setae posteriorly. One or two at the sides are much longer and there is a row of four short setae on the surface. Sternite 6 undivided, broadly triangular with the apex anteriorly and straight posterior margin which bears a similar marginal row as sternite 5. A transverse row of short setae in the posterior half of the surface. Sternite 7 broadly rounded with a row of long setae posteriorly



Figs. 40–43. *Basilia seminuda* n.sp. 40, posterior margin of sternal plate of thorax. 41, female, abdomen, dorsal. 42, female, abdomen, ventral. 43, female: (*a*) genital plate, (*b*) anal sclerites, (*c*) eye.

with a gap in the middle and some short setae near the marginal row on the surface. Anal sclerite small, drop-shaped, with two long setae. Genital plate convex posteriorly with about twelve long setae and minute hairs.

Male unknown.

A single female from unidentified host. Simla, N.W. Himalaya. (Dodsworth) (Rothschild collection).

Basilia seminuda resembles somewhat *Penicillidia peali* Scott in the bareness of the dorsum behind tergal plate 2, but differs in a number of chaetotactic characters. *P. peali* is also known from a single specimen only and belongs to the genus *Basilia* to judge from the description and the illustrations. Scott also mentions the pigmentation of the eyes and recognizes its similarity to *B. fletcheri*.

(14) **Basilia pudibunda** Schuurmans Stekhoven, 1941

A male and a female of this species were found in the Rothschild collection under the name *Listropoda comptothrinax* n.sp. (types) det. Speiser. It has apparently not been published. Although this species does not strictly belong to the *bathybothyra* group and is more closely related to the European *Basilia nattereri* and *B. nana* in most characters, it is described here, as it is intermediate in some characters between the *bathybothyra* and *nattereri* groups of Basilia.

Description. Length 1·5 mm. Colour yellowish. Very feebly sclerotized.

Head laterally compressed, anterior dorsal margin forming an angle with two setae at each side. Eyes with moderately wide posterior opening and two clearly separated ocelli. Labella of the labium slightly longer than the theca. Palps with very long terminal seta.

Thorax. Sternal plate wider than long. Length to width = 4 : 5. Median sternal suture indistinct in the middle half. Oblique sutures forming an angle of 95 to 100°. Dorsal pattern as in the other species of *Basilia*. About twelve notopleural setae. At the posterior margin of the sternal plate a long seta laterally, a shorter one towards the middle and a number of short ones between them. Legs short, tibiae scalpel-shaped, about three times as long as wide, with three rows of long setae in the distal half of the ventral surface.

Abdomen female (Figs. 44, 45). Tergite 1 widely rounded, with two groups of about seven long setae with a small gap between them in the middle. A row of five to six short setae from the lateral end of the marginal rows towards the base of the tergite. In the anterior part of the surface two groups of about ten minute hairs. Tergal plate 2 square with the posterior margin forming an angle with the apex anteriorly and an incomplete median line of division beginning at the apex of the posterior angle. Two to three long setae at the posterior margin of each half and a row of short spines. A small sclerite at the lateral posterior angles on which stand two to three long setae and some short spines as in *B. nattereri*. The exact form of this sclerite could not be made out as the tergal plate is distorted. A transverse group of short spines in the middle of the plate, leaving an anterior and a posterior stripe bare. Tergal plate 3 divided into two rectangular plates which touch in the median line. These are bare on the surface and bear 3 long setae and a row of short spines at the posterior margin. Anal segment

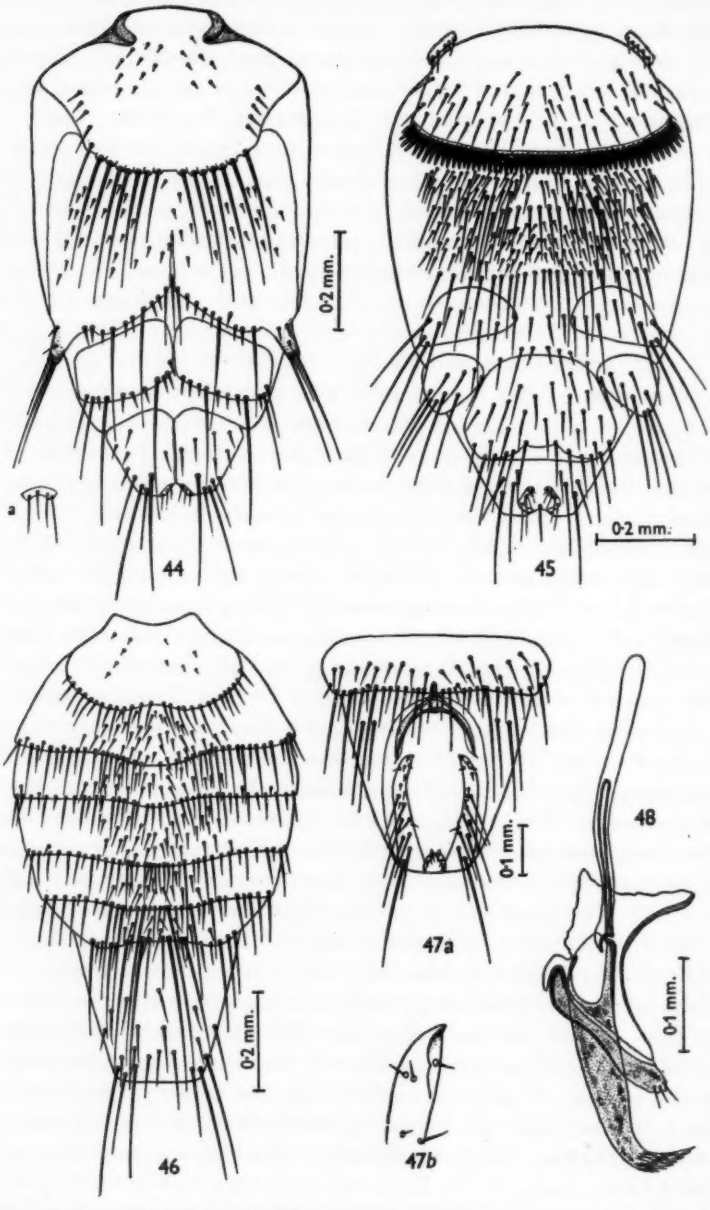
short, conical, with long setae posteriorly and some short setae dorsally. Sternite 1+2 very short, rounded anteriorly, with a ctenidium of fifty-five rather short and thick teeth and three rows of short setae on the surface. Postspiracular plate narrow, parallel-sided, with a longer seta at each end and shorter setae between them. Sternite 3 with a marginal row of moderately long setae. Surface thickly covered with rather long setae which stand more densely in two groups at the sides of the middle. Sternite 4 with a similar marginal row and four to five rows of short setae on the surface. Sternite 5 with two broadly rounded plates laterally which are widely separated. Four long setae in the lateral posterior corners and a row of five to six shorter setae across the middle of the plate. This row continues between the plates where there are three to four such setae. Sternite 6 is deeply concave posteriorly. It consists of two similar but smaller plates than those of sternite 5, still farther apart, and six setae between the plates near the anterior margin of sternite 7. This is triangular and wedged in between the plates of sternite 6. It bears two groups of five long setae at the lateral parts of the posterior margin with a wide concave gap between them. Three rows of shorter setae on the surface. Genital plate concave posteriorly with four setae, rather like that of *B. nana*. Anal sclerite indicated by a single seta.

Abdomen male (Figs. 46, 47). Tergite 1 short, rounded posteriorly with a row of short setae posteriorly, bare on the surface except for some minute hairs near the base. Tergite 2 broad, with a row of moderately long setae alternating with short ones laterally. A group of short spines in the middle of the surface. Tergites 3 to 6 narrow with similar marginal rows and groups of short spines in the middle, which become less numerous on the posterior tergites. Two long setae near the middle of the marginal row of tergite 4, two very long and two long setae on tergite 5 and four very long and two long setae on tergite 6. Anal segment conical, with long setae posteriorly and shorter setae in the posterior half of the dorsum. Sternite 1+2 as in the female, but with a ctenidium of only forty-four teeth. Sternites 3 and 4 narrow with marginal rows of moderately long setae and three to four rows of short setae on the surface. Sternite 5 not much longer than sternite 4 with a marginal row of six long setae and shorter setae between them. One or two rows of shorter setae on the surface. There is no row of short spines in the middle of the posterior margin as in most other species of *Basilia*.

Genitalia (Fig. 48). Basal arc broadly rounded, with short anterior process. Claspers thin, curved, not much tapering, with a short point at the tip. A long seta dorsally near the base and a double row of shorter setae on the dorsal surface beyond the middle. Phallobase conical, concave dorsally. Aedeagus straight, tapering, with a long upwards turned tip which ends in a fringe of scales or teeth. Apodeme very narrow. Parameres slipper-shaped with a rounded tip which bears a few short hairs.

Material examined. 1 ♂, 1 ♀ from *Vespertilio adversus* var. *horsfieldi*, Siam. (Rothschild collection.) Types in the British Museum (Nat. Hist.).

This species is of particular interest as it forms a connecting link between the European *B. nattereri* and *B. nana*, in which the second tergal plate of the abdomen of the female is square, and the forms of the *B. bathybothyra* group in



Figs. 44–48. *Basilia pudibunda*. 44, female, abdomen, dorsal, and (*a*) genital plate. 45, female, abdomen, ventral. 46, male, abdomen, dorsal. 47, male: (*a*) sternite 5 and genital area, (*b*) tip of clasper. 48, male, genitalia.

which the 2nd tergal plate is divided in the middle and has well-developed posterior processes. Tergal plate 3 is divided in *B. pudibunda* while it is undivided in the European forms. The structure of the genitalia resembles that of *B. nattereri* rather closely.

(15) **Basilia daganiae** Theodor & Moscona, 1954

This species was originally described from Palestine as a subspecies of *B. bathybothyra*, but it is of the same systematic rank as the other forms described here as species. *B. daganiae* resembles *B. majuscula* in the form of the female abdomen, except for the shorter anal segment and the much reduced anal sclerite. In the male it differs from *B. majuscula* in the form of the second tergite of the abdomen which is uniformly rounded, in that the median setae do not form the group characteristic for *B. majuscula* and are not much longer than the lateral setae, in the presence of the very long setae of the marginal row already on tergite 3, in the arrangement and number of the spines on sternite 5 and particularly in the form of the parameres which resemble those of *B. pumila*.

A single male from Mombasa, Kenya, resembles *B. daganiae* closely. It differs in the number of the spines on sternite 5 of which there are only nine as against thirteen to sixteen in *B. daganiae*. They are arranged in a single irregular row. The aedeagus is less curved and broader at the base. There are only minor chaetotactic differences. More material of both sexes will have to be examined before the status of this form can be established definitely.

Material examined. *Palestine*, Dagania. *Pipistrellus kuhlii*. (2 ♂, 4 ♀) (Palmoni). *Turkey*, Adana. (1 ♂) (Rothschild collection). *Cyprus*, Limassol. *P. kuhlii*. (1 ♂, 3 ♀) (Mavromoustakis). *Cyprus*. (1 ♂) (Lord Lilford). *Egypt*, Simbillawein, Dakhlia Prov. *P. kuhlii*. (2 ♂, 1 ♀) (Tomich). *Kenya*, Mombasa. *Cardioderma cor*. (1 ♂) (Wilson).

(16) **Basilia ansifera** n.sp.

Two specimens from Liberia in the Rothschild collection were labelled with the above name by Speiser and designated as a new species but apparently not published. Scott also examined specimens of this species from Nigeria and recognized it as a new species, but did not publish it.

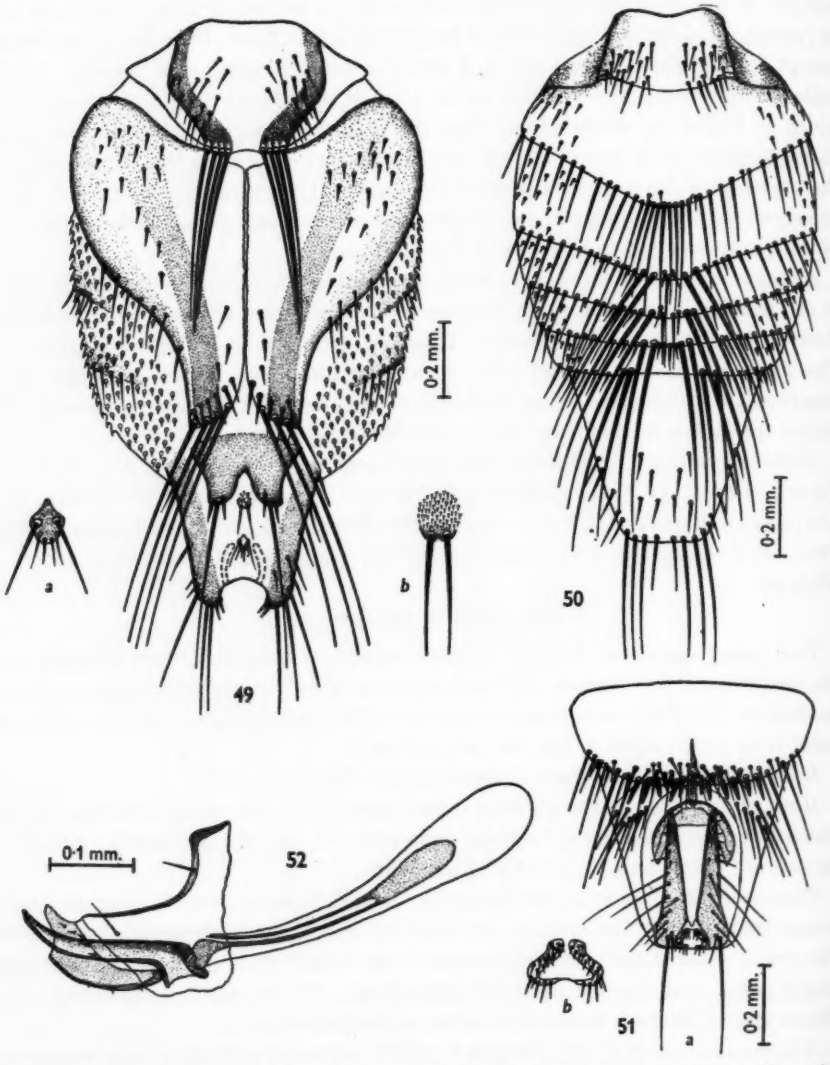
Description. Length 2 mm. Colour light yellowish brown.

Head. Four setae at the anterior dorsal margin and two setae between the eyes which have a pigmented cylindrical base and two well-defined ocelli. Labella of the labium about half the length of the theca.

Thorax. Sternal plate wider than long. Length to width = 5 : 6. Sternal median suture indistinct in the middle. Oblique sutures forming an angle of about 90°. The row of setae at the posterior margin of the sternal plate consists of about eight longer setae and shorter ones between them. Six to eight notopleural setae. Tibiae scalpel-shaped, about four times as long as wide.

Abdomen female (Fig. 49). Tergite 1 with two groups of three to five (rarely six) very long setae, with a concave gap between the two groups and two groups of short spines in the lateral parts of the surface, which are markedly longer

posteriorly. Tergal plate 2 heart-shaped with rather long posterior processes which bear three to five (rarely six) very long and thick setae, which are only slightly longer but much thicker than those of tergite 1, and a row of six to eight short spines. Two broad pigmented stripes run from the posterior processes to the shoulders. Short spines mainly in the anterior lateral parts of the surface and a few along the posterior part of the median line. The middle of the tergal plate is bare. A row of five to seven rather short setae along the posterior lateral



Figs. 49–52. *Basilia ansifera* n.sp. 49, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 50, male, abdomen, dorsal. 51, male: (*a*) sternite 5 and genital area, (*b*) anal frame. 52, male, genitalia.

margins. Tergal plate 3 with two long posterior processes which bear one or two long setae and two short spines. Anal segment rather short, but longer than wide, nearly parallel-sided, bare dorsally, with short setae laterally and some long setae and some short spines at the anal processes. Pleurae covered with minute spines. Sternite 1 + 2 wide, with a ctenidium of sixty to seventy long teeth (eighty in one specimen) and about six rows of short spines on the surface, which are markedly stronger laterally. Postspiracular plate wide, with three long setae towards the spiracle and two to three minute spines along the posterior margin. Sternites 3 and 4 with uniform marginal rows of rather short setae. Three to four rows of short setae on the surface of sternite 2 and four longer setae in the posterior row of the surface. Sternite 4 bare on the surface except for a few short spines at the sides. Sternite 5 with two narrow sclerites laterally which nearly reach the median line, a marginal row of moderately long setae which are longer laterally and one to two rows of short setae on the surface. Sternite 6 consists of two broad, triangular plates with marginal rows as on sternite 5 and two or three rows of short spines on the surface. Sternite 7 rounded posteriorly with a row of long setae at the posterior margin with a wide gap in the middle and two or three rows of short setae in the lateral posterior parts of the surface. Two long and thin setae in the posterior row of the surface. Anal sclerite drop-shaped with two longer setae at the widest part and four or five short setae posteriorly. Genital plate small, rounded, with minute hairs and two to four setae posteriorly.

Abdomen male (Figs. 50, 51). Tergite 2 triangularly produced posteriorly with a marginal row of moderately long setae and a few short spines and a group of eight to ten longer and more closely standing setae in the middle. Surface bare except for some short setae in the lateral posterior corners and a few isolated spines in the middle in some specimens. Tergite 3 also slightly produced in the middle. Tergites 3 to 6 with marginal rows of rather short setae which are shorter on the posterior tergites and a few short spines. Two to four very long and thick setae near the middle of the marginal rows of tergites 3 to 6. The long setae stand farther apart and there is a wide gap in the marginal row on tergite 6. Surface of tergites 3 to 6 bare except for some minute spines at the sides. Anal segment conical with long setae posteriorly, short setae at the sides and on the posterior part of the dorsum. Sternites 3 and 4 narrow with uniform marginal rows of moderately long setae and three to four rows of short setae on the surface of sternite 3 and one row, which is double at the sides, on sternite 4. In this row stand six setae which are much longer than those of the marginal row. Sternite 5 longer than 4, trapezoid, with an irregular single or sometimes double row of fifteen to twenty-four short spines in the middle of the posterior margin. The spines of the posterior row are longer and more widely spaced laterally. Some long setae at the side of the posterior margin and a row of long and short setae anterior to the rows of spines.

Genitalia (Fig. 52). Basal arc rounded, with long anterior process. Claspers rather short, slightly curved, with the usual dorsal long seta near the base and a dorsal row of shorter setae. Phallobase concave dorsally with two setae near the base and two others apically. Aedeagus straight and of nearly equal width in

the basal two-thirds, curved and tapering to a point in the distal third. Apodeme with a narrow end-plate. Parameres with a long, slightly curved distal process, a blunt ventral process and a shallow concavity between them. A row of minute hairs in the distal part of the dorsal edge.

Distribution. Mainly West Africa, Congo, eastwards to Lake Victoria and Lake Nyasa, nearly all between 15° latitude north and south.

Material examined. West Africa: Gambia. Eptesicus rendalli. (1 ♂, 1 ♀) (Rendall). *Sierre Leone*, Njala. *Pipistrellus minusculus.* (3 ♂, 1 ♀) (Hargreaves). *Eptesicus tenuipinnis.* (1 ♀) (Jones). Daru near Seghwema, Yileh, Benthe Distr. *Pipistrellus stampflii.* (1 ♂, 3 ♀) (Bequaert). Makeni, *P. stampflii.* (1 ♀) (Jones). *Liberia*, Du River. *P. stampflii.* (2 ♀.) Beppo's Town. *P. stampflii.* (5 ♂, 8 ♀) (Bequaert). Harbel. (3 ♀, 2 ♂) (Beatty). Begwar, Bassa. (1 ♂, 1 ♀) (Bunting) (Rothschild collection). *Ivory Coast.* Cosru, Abidjan. *Pipistrellus* sp. (2 ♂, 2 ♀) (Aellen). Tiegba, *P. nanus* and *Tadarida pumila.* (1 ♂, 5 ♀) (Rahm). *Gold Coast*, Bawku. *Scotophilus* sp. (1 ♂, 1 ♀) (Cansdale). *Dahomey.* (3 ♀) (Bouet). Porto Novo. *P. nanus.* (3 ♀.) *Nigeria*, Ibadan. 1 ♀. *P. culex.* (1 ♂, 2 ♀) (Lean). *Belgian Congo.* Flandria. (2 ♀, 1 ♂) Bamania. (1 ♂, 1 ♀) (Hulstart). Bikoro. (2 ♂, 2 ♀) (Schouteden). *East Africa: Nyasaland*, Chiromo. (1 ♂) (Wood). *A.E. Sudan*, Gondokoro. *Eptesicus rendalli.* (1 ♀.) Torit. *Pipistrellus* sp. (1 ♂). *Pipistrellus helios.* (2 ♂, 4 ♀) (Owen). Types in the British Museum (Nat. Hist.).

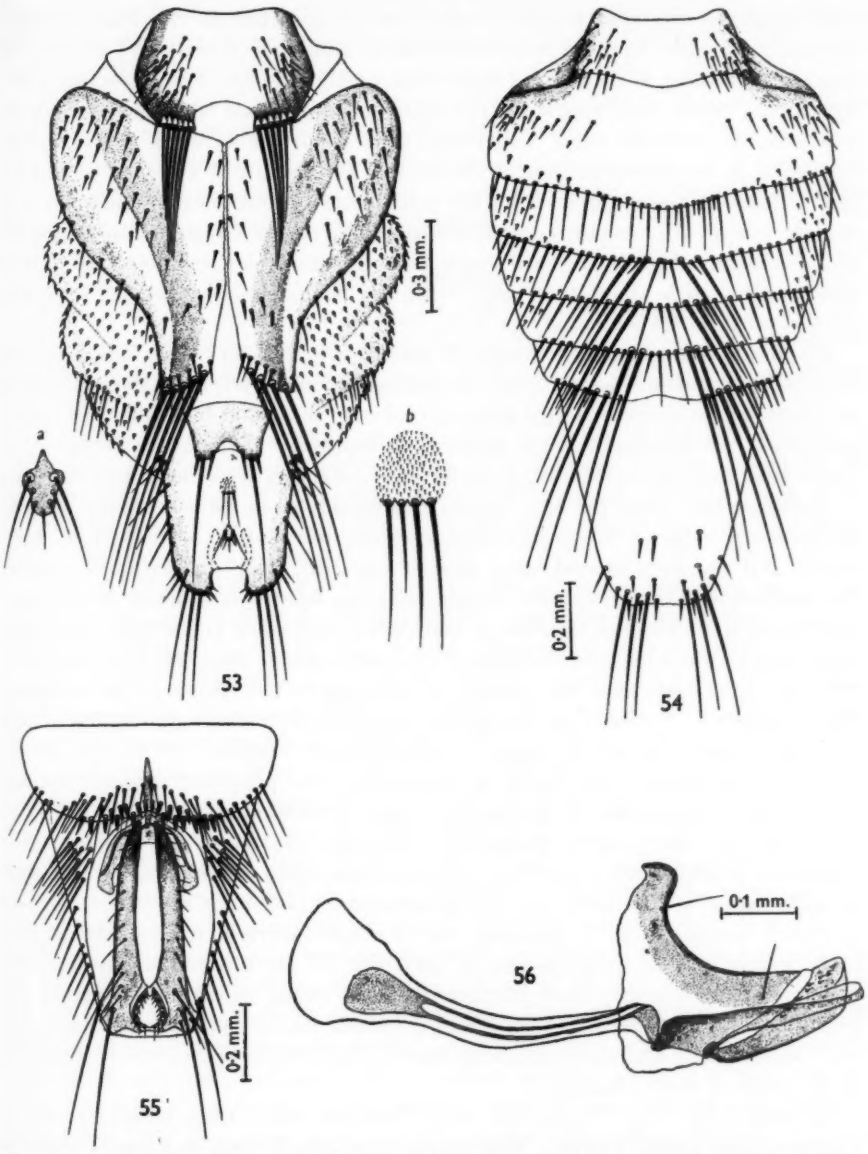
(17) **Basilia robusta** n.sp.

Description. Length 2·2 to 2·5 mm. Colour brown, more heavily sclerotized and more broadly built than *B. ansifera.*

Head as in *B. ansifera.* Eyes with wide posterior opening, ocelli rather flat, not well separated. Labella of labium very short, about one-third of the length of the theca.

Thorax. Sternal plate markedly wider than long. Length to width = 3 : 4. Median sternal suture narrow, distinct, except for a short part in the middle. Oblique sutures forming an angle of about 100°. Posterior margin of the sternal plate with a row of about ten longer setae alternating with short ones. Two lateral setae very long. Mesonotum broad, ten to twelve notopleural setae. Legs as in *B. ansifera.*

Abdomen female (Fig. 53). Tergite 1 with two posterior processes which bear as a rule four to five moderately long setae. In some specimens three, six or even seven such setae. A wide concave gap between the two groups of setae. Two groups of short setae, which are markedly longer posteriorly, in the lateral parts of the surface. Tergal plate 2 heart-shaped, with wide, truncate posterior processes which bear four to five very long and thick setae. The setae of tergite 1 are about half as long as those of tergal plate 2 in the Abyssinian specimens and two-thirds of their length in the Uganda and Congo specimens. Near the long setae a row of five to seven long spines. The surface of the plate is covered with short spines except for a broad stripe parallel to the median line in each half. At the base of the posterior processes four to five rather long spines. Five to six setae at the posterior lateral



Figs. 53–56. *Basilia robusta* n.sp. 53, female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate. 54, male, abdomen, dorsal. 55, male, sternite 5 and genital area. 56, male, genitalia.

margins which are not longer than the long spines of the posterior processes. A broad pigmented stripe from the posterior processes to the shoulders. Tergal plate 3 with two broad posterior processes which bear one or two long setae and one or two short spines. Pleurae covered with minute spines. Anal segment long, bare dorsally, with longer and shorter setae at the sides and long setae and some

short spines at the anal processes which are longer than in the other species. Sternite 1 + 2 wide, rounded anteriorly with a ctenidium of sixty-five to seventy long teeth and five to six rows of short setae on the surface. Postspiracular plate broad with two to four long setae towards the spiracle and three to four minute spines at the posterior edge. Otherwise, ventral surface as in *B. ansifera*. The long setae in the posterior row of the surface on sternite 3 are about twice as long as those of the marginal row. The sclerites of sternite 6 are rectangular and sternite 7 is broadly rounded. Anal sclerite small and of irregular form, broader in the middle, which bears two longer setae and four shorter setae posteriorly. Genital plate with about four long setae, the field of minute hairs not clearly marked off from the surroundings.

A female specimen from Bitshumbi, Lake Edward, Belgian Congo, is exceptional in the large number of setae. There are six and seven on tergite 1, five and six at the posterior processes of tergal plate 2, four long setae and four spines on tergal plate 3 and seventy teeth in the abdominal ctenidium. The setae of tergite 1 are longer than in other specimens, $\frac{3}{4}$ the length of the long setae of tergal plate 2.

Abdomen male (Figs. 54, 55). Tergite 2 uniformly rounded posteriorly without the posterior bulge of *B. ansifera*. The marginal row consists of moderately long, widely and regularly spaced setae alternating with short spines in the middle. The median setae are not much longer than the lateral ones, as in *B. ansifera*. A group of short setae at the sides of the surface and a few in the middle forming an irregular transverse row. Tergites 3 to 6 with similar marginal rows and with four very long setae near the middle. A wide gap in the middle of the marginal row of tergite 6. Surface bare except for ten to twelve minute spines at the sides of tergites 3 and 4, a few on tergite 5, tergite 6 bare. Tergites 3 to 6 very narrow. Anal segment rather long, nearly as long as the four preceding tergites, slightly conical, with short spines in the posterior part of the dorsum and at the sides and long setae and short spines posteriorly. Sternite 1 + 2 as in the female with a ctenidium of fifty to fifty-five very long and thick teeth. Sternite 5 narrow, with an indentation in the middle of the hindmargin and a double row of twenty-two to twenty-seven spines. A posterior row of about twelve to fourteen longer and more widely spaced spines begins at each side of the median indentation and reaches farther laterally than the anterior row, which consists of about ten to twelve shorter and more closely standing spines. Some setae among the spines and a row of long and short spines anterior to the rows of spines which continues at the sides of the hindmargin.

Genitalia (Fig. 56). Basal arc wide, rounded, with long anterior process. Claspers long, slightly curved, with a long seta dorsally near the base and a row of shorter setae on the dorsal surface. Phallobase strongly concave dorsally with two setae near the base and two more apically. Aedeagus nearly straight, with a strong dorsal sclerotization and blunt, truncate tip. Apodeme with wide end-plate. Parameres with truncate distal end. A double row of eight to ten minute hairs along the dorsal edge.

This species differs from *B. ansifera* in the male in the form of tergite 2, the shape of sternite 5 and its armature of spines, and mainly in the genitalia. In the

female the differences are more of degree, in the relative length and number of the setae, but also in the form of tergal plate 3.

Distribution. East Africa.

Material examined. Abyssinia, Harar. *Pipistrellus nanus.* (2 ♂, 4 ♀) (Kristensen) (Rothschild collection). *Uganda*, Bubembe Island, Lake Victoria. (8 ♀, 3 ♂) Type series. (Pitman.) *Belg. Congo*, Bithshumbi, Lake Edward. (1 ♀) (De Witte). Uvira, Kivu. *Ephesicus tenuipinnis.* (20 spec.) (Leleup). *Southern Rhodesia*, Odzi Distr. *Pipistrellus kuhlii.* (1 ♂) (Miller).

(18) **Basilia meridionalis** n.sp.

Description. Length 2·5 mm. Colour brown, strongly sclerotized.

Head as in *B. robusta.* About eight setae on the genae. Eyes with two clearly marked ocelli and wide, pigmented base. Labella of the labium less than half the length of the theca.

Thorax markedly wider than long. Length to width = 3 : 4. Oblique sutures forming an angle of about 100°. Median sternal suture distinct except for the middle third. A row of eight long setae and short ones between them at the posterior margin of the sternal plate. Mesopleural sutures wide. Ten notopleural setae. Legs as in *B. robusta.*

Abdomen female (Fig. 57). Tergite with two posterior processes which bear three to four short and thick setae and one or two short spines. A wide and deep concavity between the two groups of setae. These setae are much shorter than in the other species, less than a third of the length of the long setae of the posterior processes of tergal plate 2. This is broadly heart-shaped with wide, rounded posterior processes which bear three very long and thick setae and a row of nine to ten long spines. Surface covered with short spines anteriorly and longer spines towards the posterior processes. A row of five to six rather short setae at the lateral posterior margin. A broad pigmented stripe from the posterior processes parallel to the median line. Tergal plate 3 broad, with two broad and short posterior processes which bear a long seta and two short spines. Anal segment long, pigmented, conical, quite bare dorsally and laterally, except for one or two short spines near the long and pointed anal processes. These bear two or three long setae and several longer and shorter spines at the tip. Sternite 1 + 2 large, with a ctenidium of sixty-six very long teeth and six to eight rows of short setae on the surface. Postspiracular plate curved with three long setae towards the spiracle and two short spines at the posterior margin. Sternite 3 with uniform marginal row of moderately long setae and three to four rows of short setae on the surface. Six long setae on the posterior row of the surface. Sternite 4 with a similar marginal row and a single row of short setae on the surface which becomes double and triple at the sides. Sternite 5 with two narrow triangular plates which stand obliquely and do not reach the median line. Each plate with a marginal row of moderately long setae of which two at the sides are much longer than the rest and a row of short setae on the surface. No setae between the plates. Sternite 6 consists of two broad, rectangular plates with a marginal row of moderately long setae and two longer setae laterally and three to four rows of short setae on the

surface. Sternite 7 broadly rounded with a row of long setae at the posterior margin which become shorter towards the middle. There is a short gap in the middle of the row. A group of short spines in the posterior lateral corners and two long setae in the posterior row of the surface. Anal sclerite bean-shaped with

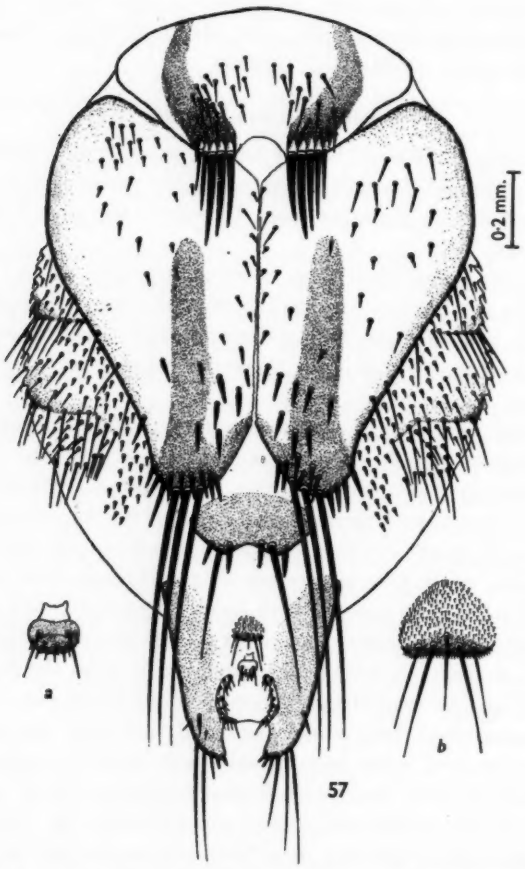


Fig. 57. *Basilia meridionalis* n.sp. Female, abdomen, dorsal: (*a*) anal sclerite, (*b*) genital plate.

a short, truncate anterior process, with two longer setae anteriorly and eight short setae posteriorly. Genital plate transversely elliptical with two long and three short setae posteriorly and covered with minute hairs.

Male unknown.

Material examined. A single female from South Africa without exact locality and host.

Distribution

The genus *Tripselia* has hitherto been considered to consist of a single species only. As shown in the present paper, it contains five or six species, and several Australian species will almost certainly prove to belong to this genus. Most of the species occur in the Oriental region and in Australia.

Only a single species, *Tripselia blainvillii*, is widely distributed in Africa and a subspecies occurs in the Oriental region. *T. blainvillii* has been found in Africa between 10° latitude north and south and in the Assumption Islands. The only specimen found outside this area is the type from Mauritius. It has not yet been found in Madagascar. The Indian specimens resemble the African form closely and are considered as a separate subspecies. Another closely related species, *T. aequisetosa*, occurs in Africa in the area of distribution of *T. blainvillii*, but more rarely. Only a few specimens from West Africa and one from the Belgian Congo have been examined so far. Its distribution thus coincides with that of *T. blainvillii* in part. It has been found so far only on *Taphozous* (*Saccolaimus*) *peli*, a species closely related to *T. mauritianus* but apparently restricted to the tropical rain forest.

Basilia bathybothyra, which has been considered hitherto as a single species distributed throughout the Oriental and Ethiopian region, has been shown to be a group of about ten different species, not one of which occurs in both the Ethiopian and the Oriental regions. *B. ansifera* is distributed mainly in tropical West Africa, in the Belgian Congo eastwards to Lake Victoria and Nyasa. *B. robusta* has been found in East Africa from Abyssinia to Southern Rhodesia and another species, *B. meridionalis*, occurs in South Africa. *B. daganiae* is mainly East Mediterranean. It has been found in Dakhlia Province in Egypt and another specimen, or a closely related form, has been found in Mombasa, Kenya. The Oriental species show a rather restricted distribution, but the material available is not sufficient to define their distribution accurately. *B. pumila* occurs mainly in Ceylon and specimens have been examined from Helwak, south of Bombay, and from Madras. *B. majuscula* has been found in Java and Sumatra and near Bombay. Both these species are thus probably widely distributed in India. *B. punctata*, a species closely related to *B. majuscula*, has been found in Ceylon and in Mysore in Southern India. *B. fletcheri* is known from Bombay, Navapur and Coimbatore. As the type specimen has been lost the status of *B. bathybothyra* from Calcutta cannot be cleared up until new material is available.

Host-parasite specificity

T. blainvillii is mainly a parasite of *Taphozous mauritianus* (forty-two specimens in ten lots). Only a few specimens have been found on other bats. *T. aequisetosa* was found only on *Taphozous* (*Saccolaimus*) *peli*. The specimens from Ceylon were found on *Taphozous longimanus* and *Taphozous* (*Saccolaimus*) *saccolaimus*. The type of *Tripselia amiculata* from Calcutta was found on *T. longimanus* and the specimens from Calcutta examined were also found on this species. The group of

T. blainvillii thus seems to be parasitic mainly on the genus *Taphozous*. The other species of *Tripselia* described were found on species of *Pipistrellus* and *Scotophilus* and *T. falcozi* on species of *Chalinolobus* and *Eptesicus*, but there is too little material at present to define their host range.

It is of interest to note that the distribution of *T. blainvillii* in Africa is much narrower than that of its host, *T. mauritianus*, which occurs throughout tropical Africa, South Africa and the Madagascar region. As stated above, *T. blainvillii* has been found so far only in a zone 10° north and south of the equator, although a great number of Nycteribiidae from South Africa and the Northern part of East Africa was examined. A similar situation has been found in the case of *Cyclopodia greeffi* and its host *Eidolon helvum* and in some Palaearctic species, where some of the host species are distributed from Europe eastwards throughout Asia, while the Nycteribiidae are restricted to the Western part of the distribution of their hosts.

The group of *Basilia bathybothyra* shows a clear specificity for the genus *Pipistrellus* both in Africa and in the Oriental region. Of thirty-three lots examined, twenty-seven were found on species of *Pipistrellus*, one on *Scotophilus* sp., three on *Eptesicus* sp. and two on *Tylonycteris pachypus*, all belonging to the Vespertilioninae and more or less closely related to *Pipistrellus*. In this group also some of the host species seem to have a much wider distribution than their parasites.

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(*MS. received for publication* 4. VI. 1955.—Ed.)

STUDIES ON THE HELMINTH FAUNA OF ALASKA

XXV. THE ECOLOGY AND PUBLIC HEALTH SIGNIFICANCE OF *ECHINOCOCCUS SIBIRICENSIS* RAUSCH & SCHILLER, 1954, ON ST LAWRENCE ISLAND

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(With Plates VII and VIII and 3 Figures in the Text)

The occurrence of a species of *Echinococcus* (Rudolphi, 1801) on St Lawrence Island was noted by the writers in early 1950. Recognition of its unusual host relationships led to an investigation of the ecology of this cestode, *E. sibiricensis* Rausch & Schiller, 1954. It is the purpose of this paper to report the results of this work, with emphasis on alveolar hydatid disease in man, of which this cestode is the etiologic agent.

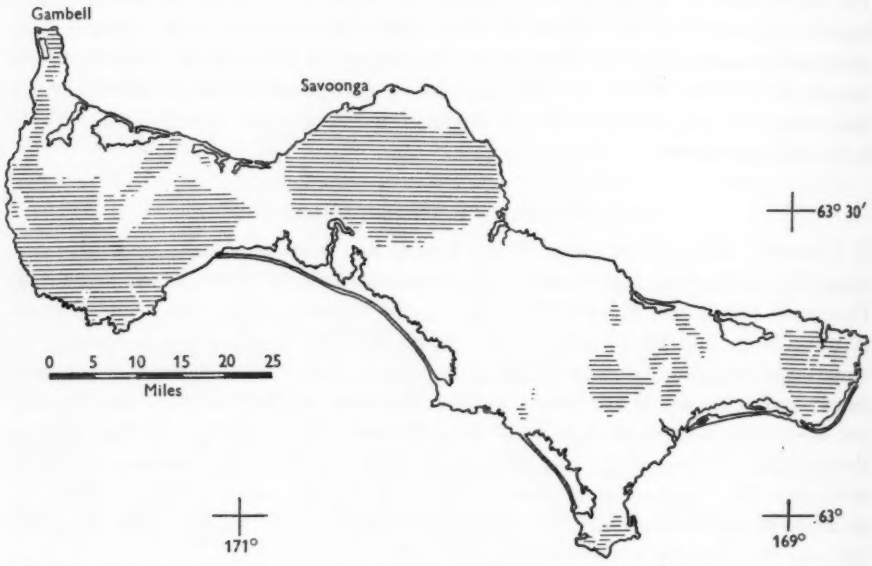
ST LAWRENCE ISLAND—GEOGRAPHY

St Lawrence Island is situated in the Bering Sea between Alaska and Siberia—about 100 miles from the nearest point in Alaska, and 38 miles off the Chukotski Peninsula, north-east Siberia (Text-fig. 1). Of volcanic origin, the island is some 90 miles long, and 20–25 miles wide. About half the land surface is covered by mountains, which range in altitude up to about 2000 ft. The climate is cold and damp, and high winds are frequent. The warmest months are July and August, but even then there are days with temperatures near freezing. In the summer the lowlands are wet, with numerous ponds typical of the arctic tundra. At higher elevations the vegetation is characteristic of the dry alpine tundra. The temperature in the winter reaches a minimum of about $-30°$ F. For more than half the year the island is surrounded by pack ice.

The mammals of the island are mainly holarctic species characteristic of high boreal regions: viz. a shrew, *Sorex tundrensis jacksoni* Hall & Gilmore; arctic fox, *Alopex lagopus* Linnaeus; ground squirrel, *Citellus undulatus lyratus* Hall & Gilmore; varying lemming, *Dicrostonyx torquatus exsul* Allen; red-backed vole, *Clethrionomys rutilus albiventer* Hall & Gilmore; tundra vole, *Microtus oeconomus innuitus* Merriam. Other land mammals occur there rarely, coming via the pack ice from Asia. Reindeer were introduced in the latter part of the century, but are now very few. Numerous dogs are kept by the Eskimo.

St Lawrence Island is inhabited by about 550 Siberian Eskimo (*Yuit*), who live in two villages—Gambell (*Sevuokuk*), slightly the larger, situated at the north-west extremity of the island, and Savoonga (correctly *Sevungak*), located about half-way down the north coast. Of the two, Savoonga has less contact with Europeans. Both villages are characterized by an assortment of wooden houses of

frame construction. About 60% of the food consumed by the Eskimo consists of flesh of sea mammals, among which the walrus is the most important. Birds and their eggs are eaten during the summer months. The people travel extensively over the island in winter for fox trapping and for hunting. Winter travel is possible only through use of sledge dogs, of which there were about 1200 in the winter of 1954–5. Outlying fishing camps, occupied during the summer months, are reached by boat. Water for household use is obtained from nearby lakes or ponds in the summer. During the remainder of the year, ice is melted. Fuel for cooking is often scarce. The dogs are usually chained near the houses, which results in a general contamination of village areas by their excreta. There is no satisfactory disposal of human wastes, since the severe climate prevents, or at best makes difficult, the application of standard methods of sanitation.



Text-fig. 1. Map of St Lawrence Island; areas of 200 ft. or more in altitude are shaded.

MATERIALS AND METHODS

Field observations on St Lawrence Island have been made intermittently during the period 1950 through 1954. During this time *Echinococcus sibiricensis* has also been maintained in the laboratory through the use of experimental animals.

Since *E. sibiricensis* does not occur on the North American continent, live-trapped arctic foxes from the Alaskan mainland have been used routinely as the final host of the cestode in the laboratory. For special purposes, arctic foxes from isolated islands (e.g. Aleutian Islands), where the life cycle of *Echinococcus* spp. could not be completed (absence of other species of land mammals), have been employed. Laboratory-reared dogs also served satisfactorily for this purpose.

For study of the larval development of *E. sibiricensis*, infective eggs were taken directly from the gravid segments of living cestodes. The latter were obtained by opening the small intestine of the final host in tap water, after which the cestodes were washed free and isolated by decantation. In determining the capacity of various species of mammals to serve as the intermediate host of *E. sibiricensis*, accurate counts of the eggs administered were not made. However, all animals used in a given experiment received approximately the same number of eggs, so that information on comparative susceptibility could be obtained. The actual number of eggs given ranged from about 400 to 2000, but larger numbers were given in certain instances. The eggs were usually introduced directly into the stomach of the experimental animals by means of pipettes of appropriate size, and the operation was performed while the animals were under ether-induced anaesthesia.

Two species of microtine rodents have been used routinely as the intermediate hosts for *E. sibiricensis* in the laboratory, as well as for controls in the attempted infection of other species of mammals. Field voles, *Microtus pennsylvanicus* Ord, were laboratory-bred, the original stock having been obtained from the eastern United States. Red-backed voles, *Clethrionomys rutilus* Pallas, do not breed readily in small cages, so these animals were live-trapped in the vicinity of Anchorage, Alaska. *Echinococcus*-infected microtine rodents have never been reported on the Alaskan mainland (Rausch, 1952).

Mammals of nearly all species considered herein have been exposed also to infection with *E. granulosus* (Batsch, 1786), the eggs of which were obtained at the autopsy of dogs to which had been fed larval cysts from the lungs of elk, *Alces alces* Linnaeus (called moose in North America). Results in all rodents thus exposed have been negative. Experimentally infected mammals were killed after passage of a desired interval of time. Following autopsy, tissues for sectioning were fixed in 10% formalin, dehydrated in ethanol, and embedded by the paraffin method. Sections were routinely stained with haematoxylin-eosin, but special stains were applied in some instances.

RESULTS

Observations concerning the adult and larval stages of *E. sibiricensis* are discussed separately.

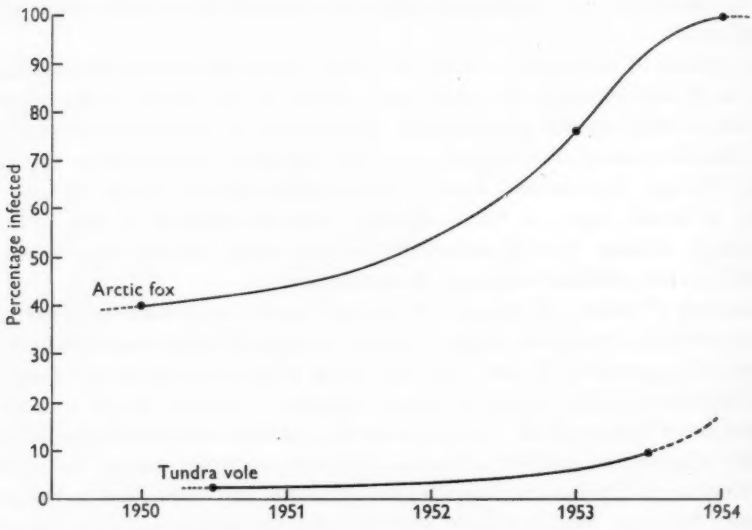
I. *The adult cestode and the final host*

Distribution. St Lawrence Island represents the easternmost limit of the distribution of *E. sibiricensis*, according to present knowledge. It is probable that the cestode was brought to St Lawrence Island from Asia by foxes carried by the pack ice (Rausch, 1953*a*; Rausch & Schiller, 1954). If this is true, *E. sibiricensis* must have been a part of the island's fauna before its colonization by man. Since the Eskimo travelled for centuries between the island and the continent of Asia, the cestode might have been introduced by means of infected dogs in relatively recent times.

A species of *Echinococcus* ecologically identical with *E. sibiricensis* occurs on Bering Island (Barabash-Nikiforov, 1938; Afanas'ev, 1941). The pack ice does not

reach Bering Island, but an indigenous race of the arctic fox is found there. The cestode was probably introduced when red-backed voles, *C. rutilus*, were intentionally brought to the island in the latter part of the last century (Stejneger, 1884; Barabash-Nikiforov, 1943; Johansen, 1950). The voles apparently originated in Kamchatka, and their presence made possible the completion of the life cycle of this cestode.

The classical alveolar hydatid disease of man, with which that seen on St Lawrence Island is clinically identical, is frequently diagnosed in central and north-eastern Siberia. According to Semenov (1954), the disease in man has been observed as far east as the lower Kolyma River region. Excepting St Lawrence



Text-fig. 2. Incidence of *Echinococcus sibiricensis* in the final and intermediate hosts over a 5-year period. Data are based on 106 foxes in 1950, 257 in 1953, and 40 in 1954.

and Bering Islands, the cestode has a wide distribution from south-central Europe eastward across European Russia and Siberia. It may well be restricted in its distribution in the palearctic region by the occurrence of certain species of microtine rodents.

Host-relationships. The arctic fox is the natural final host of *E. sibiricensis* on St Lawrence Island. The incidence of infection in this animal ranged from 40% in the winter of 1950–1, to 100% in the winter of 1954–5 (see Text-fig. 2). These values were based on the presence of fully developed strobilae only; early infections, with the scolices and beginning segments deeply embedded in the mucosa, were not detected by routine post-mortem examinations. The samples of foxes examined were taken at random from many animals trapped in early winter by the Eskimo. We have no data for the summer months, since large series of animals were not obtainable at that season.

The individual infected fox usually harbours large numbers of the cestodes. At autopsy, infections were visually evaluated as light, moderate, heavy, or very heavy. Most animals, during the time of this work, were moderately to heavily infected. Actual counts were made on representative animals, using the Sedgewick-Rafter counting chamber, and computing the total according to the average number of cestodes per cubic millimetre of sample. Infections designated as light ranged up to 25,000 cestodes; 30,000 to 50,000 was moderate; 60,000 to 80,000 was heavy. One fox, the most heavily infected animal observed, harboured in excess of 450,000 cestodes.

The cestodes attain maturity rather soon after the larvae are ingested by a canine animal. In three experimentally infected arctic foxes, we observed eggs in the faeces at 32, 33 and 33 days. The average number of eggs per gravid segment is about 200. After the production of many eggs, the adult cestodes gradually become sterile and eventually die (Rausch, 1953*b*); thus the infection of the final host is self-limiting. The production of eggs may cease as early as 3–3½ months after the date of infection, although a few cestodes may be found, in the arctic fox, as long as 7 months after infection. The greatest length of strobila, with more numerous segments, is attained by these senile cestodes. We have not determined whether duration of infection is dependent to any extent upon the diet of the host, or upon other factors less easily defined.

The adult cestodes are distributed throughout the small intestine of the final host, from a few centimetres below the stomach nearly to the caecum. The density increases posteriorly as far as the mid-ilium, where it begins to decrease. We have no observations which indicate that the final host is adversely affected by heavy infections. It seems possible that a mild enteritis might immediately follow the establishment of great numbers of immature cestodes in the mucosa, but this has not been confirmed. On St Lawrence Island, *E. sibiricensis* occurs commonly with *Taenia crassiceps* Rudolphi, 1810, *Toxascaris leonina* (von Linstow, 1902), *Uncinaria stenocephala* (Railliet, 1884), and sometimes with *Diphyllobothrium* sp.

While it has not been possible to obtain an accurate index of the status of the fox population on St Lawrence Island, estimates are based on field observations, trapping success of the Eskimo, and information provided by experienced Eskimo thoroughly familiar with the island. It has been clearly established that the density of foxes has been relatively high since 1949. During the time interval under consideration, foxes have been most abundant in the years 1953 and 1954.

Over the past six years, mouse-like rodents have comprised the major food of the arctic fox. Only the tundra vole has been important, however, since population densities of the other two species occurring here have been very low. The increase in the incidence of the adult *E. sibiricensis* in the arctic fox was directly related to the increase in the density of the vole population during the time of our observations.

The dog is also a suitable final host of *E. sibiricensis*. We have noted only a relatively low incidence of infection in dogs on St Lawrence Island, but they are, as a rule, restrained and have little opportunity to capture and eat voles. During the winter months, dogs are hampered by the snow in their efforts to capture small

rodents. In a series of thirty-one dogs autopsied in March and April 1951, 12% were found to be infected. A smaller series examined in the spring of 1950 gave negative results. Because of their value as draft animals, dogs were not often available for autopsy. We were unable to secure any of these animals in the summer months when the highest percentage of infection is to be expected. Information on this point would have been especially desirable during the summers of 1953 and 1954, when the tundra voles were abundant in the villages and relatively available to the dogs, whether the latter were chained or not. The size of the adult cestode does not differ in foxes and dogs.

It has not yet been determined experimentally whether the red fox is a suitable host for *E. sibiricensis*. The red fox is found sporadically on St Lawrence Island, but was not observed there during the course of our work. We attempted, unsuccessfully, the infection of six red foxes from the Alaskan mainland. However, all but one of these were old animals which had been in captivity for some time, so the results are not considered reliable. In one instance, an arctic fox and a red fox were fed material from the same vole; the arctic fox became infected, while the red fox did not. However, on the basis of circumstantial evidence alone, it appears that the red fox must be important in the epidemiology of alveolar hydatid disease in Eurasia. This problem is being investigated at the present time by Dr Hans Vogel, of the Tropeninstitut, Hamburg. Both the red fox, *Vulpes vulpes* Linnaeus, and the grey fox, *Urocyon cinereoargentatus* (Schreber), would come under consideration if *E. sibiricensis* became established in Canada and/or the United States.

II. *The larval cestode and the intermediate hosts*

The larval stage of *E. sibiricensis* was first collected in 1950, in the course of a routine survey of the helminths parasitic in rodents on St Lawrence Island. Although its occurrence in an unusual intermediate host (the tundra vole) was recognized, it was thought to be the larval stage of *E. granulosus* (Batsch, 1786), and was so reported (Rausch & Schiller, 1951). The possibility of the existence of a distinct species on St Lawrence Island was suggested soon thereafter (Rausch, 1951).

The tundra vole, *Microtus oeconomus*, was the only species of rodent which had any importance, during the time of our work, as a natural intermediate host of *E. sibiricensis*. Beginning at least as early as the summer of 1949, the tundra vole population increased rapidly. The greatest density was attained in the summer of 1954, when the total population exceeded any previously observed by Eskimo now living. One of us (R.R.) travelled by dog-team over the full length of the island in November 1954. Observations made at this time confirmed the opinion that the voles were abundant wherever there was suitable habitat.

With the increase in the number of voles, there was a concurrent increase in the prevalence of the larval *E. sibiricensis* (see Text-fig. 2). This was correlated with the predator-prey relationship existing between the vole and the arctic fox, and was wholly dependent upon a sustained high population of both voles and foxes.

In the summer of 1950, only 2% of 385 voles examined harboured the larval

stage of *E. sibiricensis*, but the incidence of infection had increased to 10% (320 animals examined) in the summer of 1953.* Our data on prevalence of the parasite are based on the macroscopic examination of the animals. Sectioning of the entire liver to detect early infections, as suggested by Thomas, Babero, Gallicchio & Lacey (1954) is impracticable. Foci in the liver can be recognized macroscopically as early as 48 hr. following ingestion of the eggs in experimentally infected voles. Visual identification of the larval cestode can be made with certainty by the tenth day (Rausch, 1954). No other species of taeniid cestode, whose larval stage might be confused macroscopically with that of *E. sibiricensis*, occurs on St Lawrence Island. This, however, is not true for mainland Alaska.

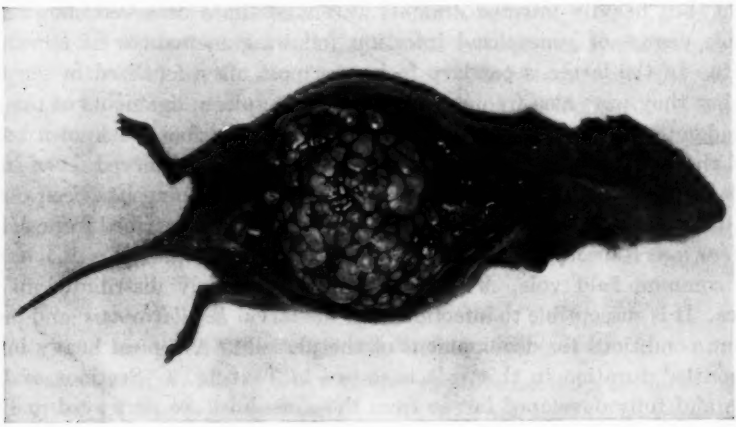
Tundra voles from St Lawrence Island did not reproduce readily in captivity, so our observations on this animal as a host for *E. sibiricensis* are limited mainly to natural infections. As would be expected, rather light primary infections were frequent, but heavily infected animals were sometimes observed showing a remarkable degree of generalized infection following metastasis of larval tissue elements. In the latter, secondary foci were most often localized in the mesenteries, but they were also frequently found in the spleen, ligaments of the uterus and bladder, and in subcutaneous locations. Primary foci are known to occur only in the liver. Pulmonary involvement has not been observed, even in those animals with generalized metastases. Histologically, nothing has been observed which differs essentially from the larval development as described from *M. pennsylvanicus* (see Rausch, 1954).

The common field vole, *M. pennsylvanicus*, is widely distributed in North America. It is susceptible to infection with the larval *E. sibiricensis*, and provides optimum conditions for development of the parasite. A typical heavy infection of 5 months' duration in this vole is shown in Text-fig. 3. Sections of 14-day, 45-day, and fully developed larvae from the same host are portrayed in Pl. VII, figs. 4–6, respectively. In addition to the laboratory-reared animals, we have also experimentally infected a number of field voles live-trapped in the vicinity of Anchorage, Alaska. Other species of *Microtus* appear equally susceptible to infection, good results having been obtained with *M. californicus* (Peale), and with a single specimen of *M. abbreviatus* Miller. These animals were live-trapped; the former near Davis, California, and the latter on St Matthew Island, Bering Sea.

Clethrionomys rutilus, a holarctic species of red-backed vole, is a suitable intermediate host for *E. sibiricensis* also. On St Lawrence Island, this vole occupies a habitat different from that of *Microtus oeconomus*, so that serious interspecific competition would seem unlikely. Nevertheless, red-backed voles were very uncommon during the time of our work, and we obtained only twenty-five of them. Of these, three adult animals captured in the summer of 1951 were heavily infected, while only one of twenty-two immature and subadult animals captured in the summer of 1953 was infected. A high prevalence of the larval stage of *Echinococcus* in *Clethrionomys rutilus* on Bering Island was reported by Barabash-Nikiforov (1938), Afanas'ev (1941), and Johansen (1950).

* Of 200 tundra voles collected on St Lawrence Island during July, 1955, 31 (15·5%) were infected.

For the experimental study of the larval development in the red-backed vole, we have used only *C. rutilus dawsoni* Merriam, a form widely distributed in mainland Alaska. The course of the larval growth in this host appears to be identical with that seen in *Microtus pennsylvanicus*. The red-backed vole tolerates infection well, and the animal survives long after the growth of the larval tissue has caused marked distortion of the body. When heavily infected, the liver can within 50 days exceed the weight of the remainder of the animal's body. In this condition the animals have an almost spherical shape, and are unable to right themselves when turned on their backs. If the number of eggs given is too great, the animals die from excessive damage to the liver within 30 days. In the laboratory, and under natural conditions, many secondary foci are seen in chronically infected animals, even though the initial infection is relatively light.



Text-fig. 3. Common field vole, showing extensive larval development in the liver, five months after experimental infection with *Echinococcus sibiricensis*.

The brown lemming, *Lemmus sibiricus* Kerr, is potentially an important intermediate host for *E. sibiricensis*. This rodent has a wide distribution in the arctic regions of Asia and North America, and is an important food resource for the arctic fox. The brown lemming is easily infected experimentally, and development of the larval cestode is identical with that observed in *Microtus pennsylvanicus* and *Clethrionomys rutilus* (see Pl. VII, fig. 7). *E. sibiricensis* could become established wherever the brown lemming and arctic fox occur together.

The muskrat, *Ondatra zibethica* Linnaeus, seems to be highly susceptible to infection, although our observations concerned only two animals. These muskrats were killed 31 and 45 days following ingestion of the eggs. The appearance of the larvae in both animals was typical of those of a comparable age in *Microtus pennsylvanicus* and *Clethrionomys rutilus*. The occurrence of the larval stage of *E. granulosus* in the muskrat was reported by Spasskii, Romanova & Naidenova (1951). These animals were trapped in western Siberia, and it is probable that the

species involved was *E. sibiricensis*. We have not attempted the experimental infection of muskrats with *E. granulosus*.

Eight mice of the genus *Peromyscus* were available for experimental infection with *E. sibiricensis*. Of these, six were subspecies of *P. maniculatus* Wagner, and there was one each of *P. boylii* Baird, and *P. truei* Shufeldt. All but one gave negative results when examined. The specimen of *P. truei*, live-trapped near Davis, California, showed a well-advanced infection when killed on the forty-fifth day. Macroscopically, the involved liver was typical in appearance, as compared with larval development in the field vole and red-backed vole. Sections showed very little leucocytic infiltration, and a minimal amount of connective tissue between the larval tissue and normal hepatic cells. The subgerminal layer was well developed, and the germinal membrane was quite cellular; scolices were numerous, and normally formed. In short, larval development was in all respects identical with that seen at 45 days in the field vole, the red-backed vole and muskrat. Additional work is needed with species of *Peromyscus*, as there may be considerable differences in degree of susceptibility in the various species of this genus.

The larval *E. sibiricensis* is capable of invading the liver of the varying lemming, *Dicrostonyx torquatus* Pallas, but it is soon overcome by the tissue reaction of the host. Animals killed on the twelfth day after ingestion of the eggs showed numerous foci which were macroscopically characteristic of an early infection. By the twentieth day, numerous foci were still present, but appeared unusually small. Sections disclosed that the lesions had a spherical shape, and there was no evidence of proliferation of larval tissue (Pl. VII, fig. 8). The subgerminal membrane was calcified, and degenerative changes had taken place in the germinal membrane as well. The zone of tissue reaction ranged from 100 to 150 μ in thickness, and was comprised of a loose stroma of connective tissue in which polymorphonuclear neutrophils were numerous. There were also scattered aggregations of lymphocytes around the periphery. Giant cells were not a prominent part of the reaction, although they have been observed frequently in other resistant species of hosts. The latest observations were made nearly 5 months (147 days) after ingestion of the eggs. At this time, calcification was evident macroscopically. Sections showed completely calcified areas surrounded by a zone of tissue reaction from 200 to 350 μ thick (Pl. VII, fig. 9). The connective tissue stroma was relatively loose, but acellular. Polymorphonuclear neutrophils were present peripherally, along with a few mononuclear cells. Focal aggregations of lymphocytes were fewer, but still present. It is concluded that the larval *E. sibiricensis* is incapable of development in the varying lemming.

Fourteen hamsters, *Mesocricetus auratus* Waterhouse, were exposed to infection, but only one had any evidence of larval invasion. The liver of this animal, examined on the tenth day, contained eight small foci which macroscopically resembled early-stage larvae. Microscopically, a lesion was demonstrated having a well-circumscribed necrotic centre containing considerable granular debris (Pl. VII, fig. 10). The area of necrosis was surrounded by a zone of polymorphonuclear neutrophils, which in turn was bounded by a thick zone of cells, mostly phagocytes. A peripheral zone, about 200 μ thick, was comprised of connective tissue, with

numerous lymphocytes and a few polymorphonuclear neutrophils. It is concluded that the hamster is not a suitable intermediate host for *E. sibiricensis*.

For the present work, the experimental infection of four species of sciurid rodents was attempted. Thomas *et al.* (1954) reported having observed well-developed larvae of *Echinococcus* in two of twelve ground squirrels, *Citellus undulatus lyratus* Hall & Gilmore, collected by them at the north-east end of the island in the summer of 1954. Of 111 squirrels from St Lawrence Island examined by us, all were free of this cestode. In order to gain some understanding of the degree of susceptibility of this squirrel, we attempted the infection of seven specimens of *C. undulatus ablusus* Osgood, trapped on the Alaskan mainland. These animals were killed on the tenth and fifteenth days. No lesions were observed macroscopically at time of autopsy, but definite evidence of larval invasion of the liver was visible in sections from only one of the five squirrels studied in detail. Many very small scattered foci of polymorphonuclear neutrophils were noted in the livers of all five animals; most of these were perivascular and involved both the interlobular and central veins. In one animal, a few rather large lesions were also present, consisting of areas of degenerating hepatic cells into which great numbers of polymorphonuclear neutrophils had migrated. A few macrophages were present, but no giant cells were observed. Larval tissue could not be identified, and the findings indicated that the larval cestodes were unable to establish themselves in this mammal. Five field voles exposed as controls had heavy infections when killed on the fifteenth day. Complement-fixation tests were performed on serum from four of the squirrels, and all gave an anticomplementary reaction.

With the above-described experiments in mind, the observations of Thomas *et al.* are difficult to explain. The squirrels examined by us were collected in the summers of 1950 and 1953, when the probability for natural infection might have been significantly less.* However, it seems that the ground squirrel is not readily susceptible, and the histological findings suggest that the larvae die early, before they migrate far from the hepatic blood vessels through which they enter the liver. Since the condition noted by Thomas *et al.* was apparently identified only by macroscopic examination, it is possible that the interpretation was erroneous. *C. undulatus* has a wide distribution in Asia and North America and might have some importance in the epidemiology of alveolar hydatid disease if it harbours the larval cestode. We expect to investigate this problem further.

One of three red squirrels, *Tamiasciurus hudsonicus* (Erxleben), was found on the fifteenth day after ingestion of ova to have many hepatic lesions. These were larger than the larvae occurring in control voles killed at the same time, and measured as much as 3 mm. in diameter. Sections disclosed larval vesicles embedded in a wide zone of degenerating hepatic cells. The path of larval migration was infiltrated by lymphocytes, and the zone immediately around the larvae contained great numbers of mononuclear leucocytes. Erythrocytes were abundant in the vicinity of the parasites. There was no evidence of any fibrosis. Some of the larvae had produced numerous exogenous vesicles, but these did not have a

* Thirty ground squirrels collected on the island in July, 1955, were negative.

typical appearance. In some there had been considerable proliferation of the subgerminal tissue, but the germinal layer was not well developed in any of them. Many of the lesions contained no recognizable larval tissue, and it was concluded that the parasites had been overcome. The complement-fixation test was negative for this animal. Evidence available indicates that the red squirrel probably cannot serve as the intermediate host for *E. sibiricensis*.

An adult specimen of flying squirrel, *Glaucomys sabrinus* (Shaw), received a large number of eggs via stomach tube. When examined on the fifteenth day, hepatic foci were evident macroscopically. Upon sectioning, these were found to consist of vesicles up to 375 μ in diameter, surrounded by a zone containing many leucocytes. The latter was comprised of necrotic and degenerating hepatic cells with many polymorphonuclear neutrophils and large mononuclear cells. Lymphocytes had infiltrated the path of larval migration from the adjacent veins. Large foreign-body giant cells were occasionally present. In most of these foci, a thin layer of larval tissue was recognized. There was no evidence of exogenous or endogenous proliferation, however, and the larvae appeared to be poorly developed. The complement-fixation test for this animal was positive. It is doubtful, however, that the larvae would have been able to persist and develop.

An adult hoary marmot, *Marmota marmota caligata* (Eschscholtz), received many eggs, and was examined on the fifteenth day. No lesions were observed macroscopically, and on sections nothing was seen suggestive of larval invasion. The complement-fixation test for this animal, however, was positive.

We attempted the infection of two young domestic rabbits. When the first was killed after 30 days, many white foci were found in the liver. Granulomatous lesions (Pl. VII, fig. 11) resembling those seen in the hamster were located on sections. Small larval vesicles, without any germinal tissue, were closely surrounded by a thick zone in which phagocytes were numerous. This in turn was enclosed by a zone of fibrosis, in which a few lymphocytes and eosinophils were present. The serum of this animal gave a strong positive in the complement-fixation test for hydatid disease. The second rabbit, killed at $2\frac{1}{2}$ months, had only a small number of healed lesions; its complement-fixation test was negative.

A 2-year-old horse was fed several thousand eggs of *E. sibiricensis*, and was killed 45 days later. Granulomatous lesions could be found throughout the liver, but nothing identifiable as tissue of the larval cestode was observed in sections. Centrally, a necrotic mass was located, containing cellular and non-cellular debris and areas of calcification (Pl. VIII, fig. 12). This was surrounded by a rather thick zone which contained a great many phagocytes and large giant cells. Peripherally, the mass was bounded by a zone of fibrosis containing lymphocytes and eosinophils, the latter abundant focally. Although negative prior to artificial infection, the complement-fixation test for hydatid disease was strongly positive at the time the horse was autopsied. The overall conclusions were somewhat obscured by the fact that this animal harboured numerous strongyles; however, it appears unlikely that the larval stage of *E. sibiricensis* is able to persist in the horse.

A herd of about 200 reindeer ranges over much of St Lawrence Island. Since these animals must frequently ingest eggs of *E. sibiricensis*, infection would seem

unavoidable if reindeer were susceptible. Since it is desired that they increase in number, these reindeer are no longer killed; however, permission to examine a few animals was granted by the Alaska Native Service and the Council of the village of Savoonga. Three old animals, one steer and two cows, were killed for study. Macroscopically, nothing suggestive of the presence of the larval cestodes was noted, excepting a few small foci observed in the liver of one old cow. Microscopically, these were found to be granulomatous lesions resembling those resulting when the larval cestodes invade the hepatic tissue of resistant species of mammals and are unable to persist. Although no reindeer have been available to us for experimental use, we believe that this species is not a satisfactory intermediate host for the cestode.

Two domestic goats, the only ruminants available for experimental purposes, were exposed to infection. The first of these animals was 7 days old when given several thousand eggs via stomach tube. When examined 15 days later, its liver was found to contain many necrotic-appearing foci averaging about 1 mm. in diameter. Compared macroscopically with the larval cestodes occurring in voles infected as controls, it was noted that these foci appeared more opaque, averaged smaller in size, and did not give the impression of being vesicular. Sections disclosed necrotic areas about 500 μ in diameter which contained degenerated hepatic cells and much granular debris, most of which was eosinophilic. Bounding this was a thin zone of palisaded epithelioid cells, which in turn was surrounded by a thick zone of mononuclear leucocytes, apparently lymphocytes. No trace of tissue resembling that of the larval cestode could be identified. There were well-defined paths of larval migration from the interlobular veins, densely infiltrated by mononuclear leucocytes. The processes of larval migration and host tissue reaction were much delayed when compared with those seen in voles. The complement-fixation test for hydatid disease gave a positive result.

The second goat, over a year old, was given a similar number of eggs in feed. When examined on the twenty-fifth day, no trace of larval migration was detected. Sections of the liver likewise gave negative results. The complement-fixation test was positive. It is possible that degree of susceptibility is influenced by the animal's age.

It is significant that Afanas'ev (1941) observed no *Echinococcus* infections in cattle, swine, or reindeer slaughtered on Bering Island. He believed that the cestode was absent from nearby Mednyi Island '. . . because its intermediate host, the vole, is also absent there'. All evidence supports the conclusion that ruminants cannot serve as the intermediate host of *E. sibiricensis*.

Two rhesus monkeys were exposed to infection, and examined after 30 days and 2½ months, respectively. In the first animal, lesions macroscopically resembling those typical of an early infection were found in the liver, although their size was smaller than expected after 30 days' growth. The central part of the lesions consisted of a rather amorphous mass of eosinophilic material with numerous phagocytic cells around the edges; no larval tissue was recognized. This mass was enclosed by a rather dense zone in which lymphocytes were abundant, along with numerous scattered eosinophils. Peripherally, the lesion was circumscribed by a dense zone

of collagenous fibres which bordered directly upon the normal hepatic cells (Pl. VIII, fig. 13). The complement-fixation test was strongly positive.

The second monkey, after 2½ months, exhibited a number of hepatic lesions of relatively large size (Pl. VIII, fig. 14). There had been much proliferation of the subgerminal layer, but there was no evidence of the germinal layer. Considerable cellular debris had been retained within the folds of the larval tissue, and this was undergoing degeneration. Directly adjacent to the larval tissue was a thin layer of rather amorphous material containing pyknotic nuclei and degenerating cells. The latter was surrounded by a thick zone of loose connective tissue, which was separated from the hepatic cells by a zone of varying thickness in which lymphocytes were abundant. The serum of this animal also gave a strongly positive complement-fixation test.*

It appears that the larva of *E. sibiricensis* is capable of survival and proliferation in the rhesus monkey, much as it is in man. However, it is probable that scolices are never produced. Long-term observations on the course of the infection in the monkey are in progress.

Negative results have been obtained in the attempted infection of mammals of species other than those discussed above, as follows: porcupine, *Erethizon dorsatum myops* Merriam, 2; kangaroo rat, *Dipodomys* sp., 1; pocket gopher, *Thomomys bottae* (Eydoux & Gervais), 1; harvest mouse, *Reithrodontomys megalotis* (Baird), 4; albino rat, *Rattus norvegicus* (Berkenhout), 37 (both young and adult animals used; pooled sera from ten subadult animals gave negative results in the complement-fixation test); albino mouse, *Mus musculus* Linnaeus, 10; domestic swine, 5 (average weight at time of exposure was 25 lb.).

ALVEOLAR HYDATID DISEASE IN MAN

The occurrence of alveolar hydatid disease in the Eskimo of St Lawrence Island was not recorded in the literature, and to our knowledge had not been recognized prior to the time of the work reported herein. Medical facilities available to the Alaskan Eskimo have been inadequate. Further, because the disease is unknown in North America, few physicians have more than a text-book knowledge of it. Human infections have likewise not been diagnosed on Bering Island, according to Afanas'ev (1941), who stated: '...up to now *Echinococcus* has not been found in man on the Komandorskii Islands, however this proves nothing because of the difficulty of its diagnosis and insufficiency of medical service on the islands.'

Most of our information on alveolar hydatid disease in the Eskimo has been obtained by indirect means through the use of the Casoni and complement-fixation tests. The application of the latter was made possible through the co-operation of Dr T. B. Magath, who has had much experience with the serologic diagnosis of cystic hydatid disease. Sera collected on St Lawrence Island were sent by air to Minnesota, and the tests were performed in Dr Magath's laboratory.

The Casoni test, using non-specific antigen (dried strobilae of *Dipylidium caninum* (Linnaeus)) of commercial origin, was first applied by us in 1950, when a

* Thomas *et al.* (1954) reported the larval cestode in one of three shrews collected. Rausch examined 13 shrews on the island during July, 1955, and found three infected.

total of 232 persons was tested, all of whom were 12 years of age or older. Reactions regarded as positive were observed in fifty-six persons. Complement-fixation tests were later performed on selected individuals.

In December 1954 the Casoni test was carried out on a total of 286 Eskimo ranging in age from 8 to more than 70 years. Of these, 260 individuals were tested with specific antigen prepared from larval tissue from an experimentally infected red-backed vole,* as well as with cystic fluid of human origin. Blood sera were collected from 308 persons for the complement-fixation test. Data on 233 persons who were given the Casoni test (using both human and vole antigens) and the complement-fixation test are summarized in Table 1. The Casoni tests were evaluated by Dr James G. Bridgens and Rausch, 20 min. after the intracutaneous injection of $\frac{1}{100}$ ml. of antigen. In nearly all cases the maximum reaction developed within this time interval.

Table 1. *Summary of Casoni and complement-fixation tests performed on 233 St Lawrence Island Eskimo in* 1954

		Tests performed								
		Casoni (alveolar antigen)			Casoni (cystic antigen)			Complement fixation*		
No. of individuals tested	Age group (years)	+	?	−	+	?	−	+	?	−
24	10 and under†	2	2	20	1	1	22	0	3	21
77	11–20	16	6	55	3	2	72	9	3	65
45	21–30	16	2	27	10	2	33	3	3	39
29	31–40	14	2	13	4	1	24	6	3	20
30	41–50	17	2	11	10	2	18	10	4	16
14	51–60‡	5	1	8	3	0	11	3	0	11
14	61 and older	8	1	5	6	5	3	4	5	5
233		78	16	139	37	13	183	35	21	177

* Fifty complement-fixation tests, not included in table, were performed on individuals on whom no skin test was made. These are tabulated as follows: 9 positive, 1 doubtful, and 40 negative.

† Youngest individual (not in table) reacting positively to the complement-fixation test is 9 years old.

‡ Not included here is one individual, aged 54, who gave positive reactions to both alveolar and cystic Casoni tests, and a strong anticomplementary reaction to the complement-fixation test.

Antigen derived from the alveolar larva produced about twice as many positive Casoni reactions as did the antigen of cyst fluid origin (*E. granulosus*). In comparing results of the Casoni and complement-fixation tests, it was found that twenty-six of the thirty-five persons for whom the complement-fixation test was positive also gave a positive Casoni reaction using the alveolar-type antigen. Only seventeen positive Casoni tests for the same thirty-five persons were obtained with the cyst fluid antigen. The Casoni test was negative with both antigens in nine of

* This was prepared by grinding in a mortar 20 g. of larval tissue in 100 ml. of normal saline, followed by incubation for 4 hr. at 37° C. The filtrate was used without further dilution. This, as well as the cystic fluid, was preserved by the addition of 1:50,000 merthiolate.

the thirty-five persons for whom the complement fixation was positive. A positive Casoni reaction (using the alveolar antigen) was obtained for fifty-two persons for whom the complement-fixation test gave negative or doubtful results.

A positive Casoni reaction with a negative-complement fixation test may be indicative of an infection which has been overcome by the tissue reaction of the host. Magath (1953) reviewed the diagnostic tests for hydatid disease, and concluded that a positive Casoni test may persist for the life of the individual. He stated (p. 223): '. . . this test cannot be used as a measure as to whether the patient is suffering from a live hydatid cyst, from a suppurative cyst, from a calcified cyst, or from the results of a former infection which is now completely obliterated.' From a review of the literature, Magath concluded that results with the Casoni test range from about 60 to 90 % reliable in indicating past or existing infections. Henschen & Bircher (1945) expressed the opinion that the Casoni test appears to be more dependable than the complement-fixation test in the diagnosis of hydatid disease. It was stated that they, as well as other workers, had observed several positive reactions in the presence of alveolar hydatid disease when fluid from the larval cyst of *E. granulosus* was used as the Casoni antigen.

Regarding the complement fixation, Posselt (1925) did not consider this test well suited to the diagnosis of alveolar hydatid disease. Since alveolar larvae from man are usually sterile and necrotic, he believed that a specific antigen could not be obtained from them. Magath (1953), however, had good results in the diagnosis of cystic hydatid disease, using cyst fluid preserved with merthiolate as the complement-fixation antigen. Positive tests employing cyst fluid antigen were obtained on sera from mammals infected by us with the larval stage of *E. sibiricensis* (confirmed by post-mortem examination). Accurate results were also obtained in man, in so far as we have been able to confirm them by indirect means of diagnosis.

Differential counts were made on blood smears of 242 Eskimo for whom data on Casoni and complement-fixation tests were available. Of these forty-one (17 %) demonstrated a degree of eosinophilia exceeding 5 %. The maximum observed was 27 %. No significant correlation could be made between the occurrence of eosinophilia and other findings indicative of active infections. Both Posselt (1925, p. 596) and Henschen & Bircher (1945, p. 216) concluded that eosinophilia is inconstant and of little diagnostic value for alveolar hydatid disease. We have not observed eosinophilia of the peripheral blood in experimental animals having advanced infections.

In addition to the data on the Eskimo, we have obtained sera from 153 military personnel who spent a year or less on St Lawrence Island. Of this total number, twelve gave positive complement-fixation tests, and ten gave doubtful results. An effort is being made to check periodically those men who have shown positive or questionable reactions, in order to observe any changes in titre. At the time of this writing, second tests have been performed on six persons, as shown in Table 2. Observations on these men will be continued as long as possible, or until negative tests have been obtained. Repeated tests have been made at intervals of 3–6 months. If active alveolar hydatid disease develops in any of these men, it

will be of unusual interest, since the time of exposure is known, and the prepatent period could be determined.

When the larval stage of *E. sibiricensis* is able to persist in the human host, it may be very pathogenic. Years may pass before signs of the disease become apparent and, under conditions existing until recently on St Lawrence Island, diagnosis and treatment were unlikely. Before the time of this work, a few

Table 2. *Results of complement-fixation tests on* 153 *military personnel*

Positive		Doubtful		Tests repeated (6)		
				2nd	2nd	2nd-3rd
No.	%	No.	%	positive	negative	doubtful
12	7·84	10	6·53	1	3	3

Eskimo underwent surgical treatment for the removal of undiagnosed masses in the liver. More recently, two persons have been treated surgically for alveolar hydatid disease at the Tacoma Medical Center, Tacoma, Washington. Detailed information on these patients, with material for study, has been made available to us. In further support of the conclusion that the disease on St Lawrence Island is identical with that in Eurasia, the case histories are summarized here.

(1) I.A., male, age 28 years. First symptoms, reported in January 1949, included epileptiform convulsions which occurred with increasing frequency. The seizures had been preceded by disturbed gait, which became constant. Severe headaches were common and there was progressive disturbance of vision. An intracranial neoplasm was suspected. Craniotomy was performed on 9 November 1950. A mass of increased resistance was removed from deep in the right postfrontal area. This tissue contained many cysts of variable size. Microscopically, there was marked infiltration by lymphocytes and plasmacytes, with perivascular fibrosis, gliosis and necrosis of tissue adjacent to the cysts. The postoperative course was difficult. The operation was followed by moderate paralysis of the left arm and leg; improvement was gradual, and there was a residual weakness of the left arm. The patient was discharged to his home, where he died in July 1951. No autopsy was performed.

(2) M.I., female, age 58 years. In 1950 an enlarging mass was discovered in the epigastrium. Exploratory laparotomy performed in Alaska on 19 March 1951 disclosed a tumour-like mass 12–15 cm. in diameter below the xiphoid process and to the right of the midline. The right lobe of the liver was involved. Treatment consisted of the withdrawal of about 250 c.c. of thick, gelatinous, muco-purulent material by means of a trocar. Streptomycin was introduced into the collapsed cyst, whose opening was closed by a purse-string suture. Recovery was uneventful.

With recurrence of the mass in the epigastrium, the patient was admitted to the Tacoma Medical Center in 1953. Laparotomy was performed on 27 May 1953, and a cyst about 15 cm. in diameter was found in the incisura between the right and left lobes of the liver, on the anterior surface. There were adhesions to the abdominal wall, and the right lobe of the liver was involved. About 500 c.c. of

thick, greenish, purulent material was evacuated, and the cyst was removed *in toto* by dissection. The thick-walled cyst was opened prior to fixation, and was 11 cm. in greatest diameter. The outer surface exhibited irregular convolutions and adherent strips of tissue. The wall of the cyst varied in thickness from 2 to 10 mm., with thicker areas corresponding to nodular elevations on the outer surface. The wall was greyish white in cross-section, with a yellowish, necrotic-appearing membrane on the luminal surface. Calcium deposits were present focally in the wall. Further sectioning of the wall revealed some areas almost cartilaginous in nature. Small cystic areas were present, with cysts, measuring 1 to 2 mm. in diameter, filled with translucent, gelatinous material. Scrapings from a small cyst exhibited laminated membranes consistent with the diagnosis of hydatid disease. No scolices were observed.

Microscopically, confluent or solitary cysts were seen at different levels of the cyst wall (Pl. VIII, figs. 15, 16). The lumena of the cysts were lined by a compact, acellular laminated layer. Adjacent to the latter, away from the lumen, the tissues were fibrosed and somewhat fragmented. This zone merged with the layer of proliferating reticuloendothelial cells in which there were occasional scattered giant cells. Lymphocytic and some neutrophilic infiltrations were noted. In the lumena of cysts, no germinal layer was observed, but there were occasional small protrusions suggestive of scolices. Remnants of liver parenchyma were attached along the outer margins of the cysts. Chronic granulomatous inflammation was extensive throughout all sections examined. Some zones were poorly cellular and had the appearance of being completely hyalinized. At other levels there was reactive fibrosis, and numerous foam cells intermingled with masses of erythrocytes, lymphocytes, eosinophils, and plasma cells. Occasional groups of fat cells were also found within the inflammatory tissue.

Recovery was uneventful. When the patient was examined by Dr Bridgens on 15 December 1954 the liver was not palpable, and the spleen could be felt only upon deep inspiration. All indications were that the infection had been extirpated.

The infection in the second patient was very similar to those reported by European and Russian workers. For comparison, descriptions of two cases are quoted from Semenov (1954). The first of these originated only a few hundred miles west of St Lawrence Island.

1. Case C-va M. 35 years; 28. vii. 49. Brought by plane from Nizhne-Kolymskii region. An objective investigation turned one's attention to a very large abdomen with a cyst visible to the naked eye in the epigastral region. The cutaneous layers are icteric, with many scratched areas and pyodermis. Palpation shows rock-like solidity of the liver protruding from under the rib arch 12–15 cm. with large protrusions on the surface. The sclerae are icteric. Ascites. Edema of the legs. As concerns heart and lungs, there are no essential changes. Casoni reaction positive.

On 12. ix. the patient was operated on under local anaesthesia. An incision of moderate size was made above the navel and the abdominal cavity was opened. Ascitic fluid of a brownish red colour was exuded under great pressure. The liver was of enormous dimensions as a consequence of the parasitic cyst which had encompassed almost the whole right lobe and partly the left (there were individual nodules about the size of a fist). The liver capsule was preserved only on the periphery of the cyst and in sections of the liver. In consequence of the inoperability of the cyst, the abdominal cavity was completely closed in layers after extraction of pieces of the tissue for histologic investigation. The patient lived for three days

after the operation and on 14. ix. there was the appearance of increased ascites and cachexia and weakening of the heart; she died. At the autopsy, the clinical diagnosis was affirmed.

2. The patient K-v.V.A., 12 years old, entered the surgical section from the city of Olekminsk on 3. vii. 45. Inasmuch as an experimental laparotomy had already been performed on the patient in the regional hospital, an attempt at a radical removal of the parasitic cyst was made. On the 1. viii. 45 day, under ether, the abdominal cavity was opened with a moderate incision from the xiphoid to the navel. Investigation disclosed alveolar echinococcus of the left lobe almost to the size of two fists, clearly visible only from the side of the lower surface of the liver. Puncture of the echinococcus cyst produced a thick matter. The operational field was isolated by shrouds; from the cyst which was of extraordinarily solid consistency was cut an oval piece. There was visible a massive little wall of the thickness of 2 cm.; in the centre of the cyst was disintegration and pus. After removal of the pus and a working over of the cavity with a five per cent formalin, the wall of the parasitic cyst was cut off with separate concentrically widening areas. In such fashion almost the entire cyst was removed, with the exception of one-third of it which was found deep; the latter was removed with a sharp spoon up to the appearance of the liver capsule. As a consequence of the beginning of haemorrhage, the cavity was temporarily tamped up. After stopping of haemorrhage, the remaining unremoved thin peripheral layer of alveolar echinococcus was cut away along with the liver capsule. On the wound of the liver were placed catgut sutures. The incision of the abdominal wall was sewed up in successive layers. In the postoperative period there was noted a sharp falling of heart activity, a collapse. Despite measures taken, the patient died toward morning.

The causative agent of alveolar hydatid disease was first recognized by Virchow (1855) as being the larval stage of a cestode of the genus *Echinococcus*. Following Virchow's observations, the specific identity of the cestode involved has been a matter of considerable controversy. Some investigators have had the opinion that, under certain conditions, the larva of *E. granulosus* develops anomalously and produces the alveolar formation; others have believed that a distinct species of *Echinococcus* is involved. The controversy need not be discussed further here, since it has been reviewed recently in detail by Dew (1953). To our knowledge, alveolar hydatid disease has not been demonstrated experimentally prior to the present work.

Although the larva of *E. sibiricensis* may frequently infect man, it rarely persists long in this host according to available information. It appears that man is an accidental, and altogether unsatisfactory intermediate host for this cestode, and larvae from the human host usually possess the following characteristics which support this conclusion: (1) growth progresses slowly; (2) the bulk of the larval mass is necrotic, with only a thin peripheral layer of viable tissue (specifically in hepatic infections); (3) the germinal layer is usually absent, and scolices are rarely produced. It might be said that man and the larval cestode are mutually pathogenic. The above-mentioned points are discussed separately.

Very rapid growth of the larval cestode is characteristic in hosts which are regarded as the natural ones (Rausch, 1954), and the infective stage is attained in a relatively short time. Rapid development is necessary for successful propagation of the cestode when short-lived animals (as voles) are involved. It is agreed by clinicians familiar with alveolar hydatid disease that human patients may remain asymptomatic for years. This cannot be explained by the fact that most human infections may derive from ingestion of only a few eggs.

The larval mass is largely necrotic in classical human alveolar hydatid disease

involving the liver, and only the cestode tissue in close apposition with the tissue of the host is able to survive. We have not observed necrosis in any experimentally infected voles, irrespective of the size attained by the larval mass or of the degree of damage to the liver in which it occurs. If such larvae were largely necrotic, the number of scolices present would be greatly reduced, and this in turn would affect the reproductive success of the cestode. It is evident that the host-parasite relationship existing between the larval *E. sibiricensis* and certain mammals which it may invade is not always favourable to the cestode, as evidenced by the kind of local tissue reaction evoked. In a favourable relationship, the parasite has become so adapted to the host organism that its presence does not cause a foreign-body-type reaction. Severe tissue reactions alone are indicative of an unnatural parasite-host relationship, and more or less comparable reactions have been observed in the varying lemming, hamster, rabbit, horse, goat and monkey—most of which are able to overcome and destroy the larval cestode. Thus, calcified areas, very dense leucocytic infiltration, numerous giant cells, and marked proliferation of connective tissue are regularly observed in the mammals listed above as unnatural hosts. A pronounced tissue reaction is not characteristic of infections in voles and other highly susceptible mammals.

From a review of the literature and other sources of information, it is evident that larvae in man rarely produce scolices. Virchow (1855) observed scolices, apparently malformed, in the case studied by him. He stated (page 92):

> Einzelne Thiere hatten ihre regelmässige, ausgestülpte Form und man unterschied an ihnen den rundlich eiförmigen Hinterleib mit einer, dem früheren Stiel entsprechenden trichterförmigen Einsenkung und den etwas breiteren und grösseren Kopf mit seinen vier Saugnäpfen und der scharf begränzten Mundgegend. Manche dieser Thiere hatten keine Spur eines Hakenkranzes und bei einzelnen, kleineren und blässeren möchte ich in der That glauben, dass sie noch ganz jugendlich waren und überhaupt noch keine Haken besessen hatten. Andere dagegen waren sehr gross und durch reichliche Anhäufung von gelbbraunen und braunrothen Pigmentkörnchen am Mund, den Saugnäpfen und dem Hinterleibe ausgezeichnet, so dass hier wohl ein secundärer Verlust der Haken stattgefunden haben könnte.

Dardel (1927) and Henschen & Bircher (1945) did not succeed in rearing adult cestodes when dogs were fed larval material of human origin. Posselt (1904), however, fed alveolar larval material from man to a recently weaned dog, whose faeces had been examined for cestode eggs, and to whom an anthelminthic had been administered. He later autopsied the dog, and reported successful infection (p. 90): 'Bei demselben wurde *zum erstenmale in vollkommen einwandsfreier Weise die Taenia echinococcus alveolaris* (*multilocularis*) in zahlreichen Exemplaren gezüchtet.' To our knowledge, the results obtained by Posselt have never been duplicated.

THE EPIDEMIOLOGY OF ALVEOLAR HYDATID DISEASE

Human infection is most likely to occur at times when voles and foxes are abundant. Under conditions existing during the period 1950–4, it is difficult to believe that any of the Eskimo could avoid ingesting eggs of *E. sibiricensis*. Both dogs and foxes are important disseminators of the eggs, but their importance changes in relation to a variety of influencing factors.

The Eskimo have direct contact with the arctic foxes during the winter, beginning about 1 December with the opening of the trapping season. After capture, the foxes are brought to the villages or to trapping camps, and all subsequent procedures are carried out in the living quarters. The animals are hung in a warm place to thaw, and are then skinned, usually by the women. During handling, eggs adhering to the fur may be shaken off and scattered about. The foxes are skinned in the Eskimo manner, by cutting around the mouth and withdrawing the body without cutting the skin. In freeing the skin from the bone of the tail, the skinner usually grasps the base of the tail in the teeth, having one hand inside the skin and the other outside. The hides are stretched and dried inside the house, where they are hung near the ceiling for greater warmth. After drying, the skins are worked until soft, and are then cleaned by beating them in fine sand or flour. The skins are sold to the village co-operative store, and are eventually sent to the continental United States for tanning and processing.

Sledge dogs may comprise an important source of human infection. When not being worked, during the summer months, the dogs are poorly fed and will eat voles and other small animals whenever possible. Although dogs are not nearly as adept at capturing voles as are the foxes, they are able to eat them frequently if unrestrained. Dogs which are chained do not often capture any voles since the latter keep to areas grown to vegetation. However, in periods of high population density, such as the summers of 1953 and 1954, the voles move about the villages and commonly are seen running on vegetation-free ground. Under such conditions, they are easily caught by the dogs.

Through custom and because of greater convenience, the dogs are chained near the dwellings. Young dogs, those unfit for work, and occasional strays may be at liberty. In 1954, there was one team of about ten dogs for every two families on the island. The villages are badly contaminated in general, with a concentration of canine and human excrement around the dwellings. There is always a risk of carrying faeces into the houses on footwear and on any implements to which dogs have had access. Harnesses and equipment used in connexion with the dogs are badly soiled, but nevertheless are repaired indoors. In unfavourable weather, dogs about to whelp or dogs in poor condition may be taken into the shelter of the storm-porch where much of the food of the household is stored. During the snow-free time of the year, eggs of *Echinococcus* can be picked up by the dogs' fur and transferred to the hands of persons working with them.

During the working season, the dogs are well fed if food is available. At this time, few voles can be captured by them because of the shelter afforded by the snow cover. In handling the dogs on the trail, gloves are never changed nor are the hands washed afterwards, or before eating, as it is so cold that such sanitary precautions are nearly impossible to observe. Water is obtained only by melting ice or snow, and fuel is not readily located.

It is evident that any dog harbouring *E. sibiricensis* is a threat to persons coming in contact with it. We estimate conservatively that there is an average of one infected dog per team; however, this will vary according to the season of the year and the density of the vole population.

Little is known about the contamination of food and water. Some vegetation is consumed unwashed when the Eskimo are away from the villages. The leaves of *Saxifraga punctata* L. and of *Salix* spp. are commonly eaten, and these are collected in places subject to contamination with the faeces of foxes. Green vegetation is preserved by freezing for use in the home, but it is possible that the cestode eggs are killed if the period of preservation is long enough.

Small ponds and other bodies of water may contain eggs of *Echinococcus* when foxes are abundant, and these might constitute a source of human infection under conditions of travel. We have successfully infected voles with eggs of *E. sibiricensis* kept in pond water at room temperature for 22 days. In the winter, the melting of ice in the home for water, without bringing the water to boiling or at least to a high temperature, might be hazardous. Ice for this purpose is cut and often stored near the house, where it is exposed to contact with excrement of dogs.

The eggs of *E. sibiricensis* are resistant to cold, and remain infective after prolonged exposure to freezing temperatures. Experimentally, we have found that some eggs remained infective after having been frozen at $-26°$ C. in a mixture of water and intestinal content of foxes for 60 days. Some eggs were still infective among a quantity exposed to temperatures as low as $-56°$ C. in the laboratory. The eggs of *E. sibiricensis* have been found to survive long periods frozen *in situ* in the carcasses of infected foxes.

Since the immediate village areas are largely free of vegetation, there is also the possibility of contamination of food by eggs in wind-borne dust. Schiller (1954) has shown experimentally that the eggs of *E. sibiricensis* may be transmitted to susceptible mammals by adult blowflies *Phormia regina* (Meigen). Blowflies of other species are numerous on St Lawrence Island, where they breed around the villages in carcasses of dogs and other animals, but it is unknown whether they have any part in the transfer of eggs under natural conditions.

CONTROL OF ALVEOLAR HYDATID DISEASE

The control of alveolar hydatid disease on St Lawrence Island will depend upon the initiation and enforcement of measures designed to control dogs and improve sanitary conditions.* Adequate precautions regarding the dogs are most important, since close association of the Eskimo and their sledge dogs is not to be avoided.

All dogs should be chained in areas which are free of vegetation and at a reasonable distance from the dwellings. No dogs should be allowed to run at large, and unwanted animals should be destroyed. The periodic treatment of dogs with an anthelminthic might significantly reduce the number of cestodes which they harbour, although this is doubtful, since the cestodes are so deeply embedded in the mucosa of the intestine. If dogs were appropriately controlled, the trapping of voles around the houses might aid in reducing the number of canine infections.

* Following our recommendations, regulations prohibiting the export of dogs from St Lawrence Island were put into effect by military authorities in 1951, and by the Alaska Department of Health in March 1953.

The handling of foxes presents many problems. Fox trapping is a basic economic enterprise in which the Eskimo engage, and serious changes cannot be made. Obviously, then, the people must be instructed as to the proper handling of these animals. A special building could be provided for the thawing and skinning so that such work would not be done in the homes.

Interruption of the life cycle of the cestode by eliminating one of its hosts is not feasible. In view of the size of the island, any measure severe enough to kill many voles would be biologically unsound and unworthy of consideration. Since the island is naturally recolonized by foxes annually, control of these animals would be impracticable. Furthermore, the economy of the Eskimo would suffer seriously if the foxes were reduced in numbers.

It is especially important to prevent the introduction of the cestode on the North American continent. This seems altogether possible if regulations already established by the territorial government and military authorities, prohibiting export of any dogs, continue to be enforced.

In so far as malignant infections are concerned, morbidity can be reduced through an enlarged medical service equipped to undertake appropriate measures of diagnosis and treatment. At the present time, surgical intervention as early as possible provides the only adequate treatment of the disease, as has been emphasized by Henschen & Bircher (1945) and by Semenov (1954). The broad experience of Semenov led him to the conclusion that radical resection of the infected hepatic lobe is the only appropriate treatment. He stated (p. 24): '...resection of the liver cannot be done on the diffused form, as well as on the alveolar nodular form if the nodules are distributed throughout the whole liver.' Among forty-two patients upon whom Semenov conducted exploratory laparotomies, the diffuse form was seen in fifteen cases, and nodules in both lobes of the liver were present in twenty-five cases. He concluded that: '...in the presence of alveolar-nodular or the concentrated form if there is observable a lone parasitic cyst a radical operation is successful in almost 3/4 of the patients.' The prognosis is always poor when the disease exists in an advanced form.

SUMMARY AND CONCLUSIONS

It is concluded that *E. sibiricensis* is the etiologic agent of alveolar hydatid disease in man. The cestode has a wide distribution in Eurasia, and St Lawrence Island apparently represents the north-easternmost extent of its range. The study of the cestode in Europe has been complicated by the co-existence of *E. granulosus*, which does not occur on St Lawrence Island. Microtine rodents, particularly *Microtus* spp. and *Clethrionomys rutilus*, are the natural intermediate hosts of this cestode, although other species of mammals, including man, are parasitized with varying degrees of success on the part of the larval cestode.

Man is not a favourable host—a view supported by the fact that normal growth and reproduction (i.e. the production of scolices) rarely occur in man, as well as by the comparative study of larval development in various species of experimentally infected animals. Although the influencing factors have not been defined, the larval cestode can become established in some persons, persist, and cause

morbidity or even death. The number of eggs ingested and the physical condition of the host may be important. There is evidence that the larvae are in many cases overcome by the tissue response which they elicit.

Human infection is most likely to occur where cultural and social conditions enforce the close association of man and dog, as on St Lawrence Island and in the northern part of the Iakut Autonomous Republic, eastern Siberia. In such regions, dogs are kept for transportation or for herding, and climatic conditions, lack of education, and poverty make difficult the application of ordinary hygienic procedures. Human infections may also be found in northern Eurasia where trapping of the arctic fox is important economically. Military personnel stationed in such regions should not be permitted to have dogs as pets, and the care of work dogs should be carefully supervised.

In southern Europe and western Russia, dogs harbouring the adult cestodes may unknowingly be kept as pets, or for use with domesticated animals. The same situation would exist if the cestode were introduced into the United States or Canada. The earlier concept that alveolar hydatid disease is caused by *E. granulosus*, or at least by a cestode whose larval stage is found in domesticated animals, is especially misleading, since the slaughter of livestock in modern abbatoirs effectively prevents access to infected offal by dogs. Dogs could ingest the larval cestode even in densely populated semi-rural areas if voles are present, so dissemination of information on the epidemiology of the disease will be important. Investigations being carried on by Dr Hans Vogel, Tropeninstitut, Hamburg, will undoubtedly produce the information essential to an understanding of the ecology of the cestode in Europe.*

In treating patients having alveolar hydatid disease, a favourable prognosis can be made only if there is early diagnosis. The population should be aware of the seriousness of infection, and of its clinical signs. Application of clinical and serologic diagnostic procedures is required, and physicians who may receive patients from regions where the disease is endemic should be adequately instructed. Radical resection of the infected hepatic lobe is apparently the only satisfactory treatment at the present time. The ease with which the life cycle of *E. sibiricensis* can be studied in the laboratory permits investigations which have not been possible with *E. granulosus*. It is especially adaptable to studies on the effect of chemotherapeutic agents on the larval stage.

The writers take this opportunity to express appreciation for the assistance rendered in the course of this work by the following persons: Dr Thomas B. Magath, Mayo Clinic, Rochester, Minnesota, who performed the complement-fixation tests and offered many suggestions; Dr James G. Bridgens, Kansas City General Hospital, Kansas City, Missouri, who rendered valuable assistance in connexion with clinical observations on the St Lawrence Island Eskimo; Dr Francis H. Fay, University of British Columbia, Vancouver, B.C., who provided detailed field observations for the summer of 1953, and made available mammals

* Since this paper was submitted, a preliminary report has been published by Vogel (*Dtsch. Med. Wschr.* **80**, pp. 931–32, 1955).

collected on St Lawrence Island; Dr Hans Vogel, Bernhard Nocht-Institut für Schiffs- und Tropenkrankheiten, Hamburg, who made available unpublished data on his investigation of alveolar hydatid disease in Germany; Miss Grace Crosson, R.N., Alaska Department of Health, who assisted whenever possible with work on St Lawrence Island; Mrs I. L. Wallace, Laboratory of Bacteriology of this Center, who rendered technical assistance; and Mrs Reggie V. S. Rausch, of this Center, who prepared all histological materials and figures. Other persons also, not specifically mentioned, are extended our best thanks. Surgical specimens were made available through the co-operation of the Tacoma Medical Center, Tacoma, Washington. The generous co-operation of the Eskimo of St Lawrence Island has done much to make this investigation possible, and we wish especially to acknowledge their contribution.

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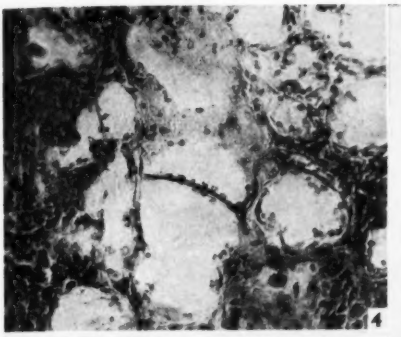

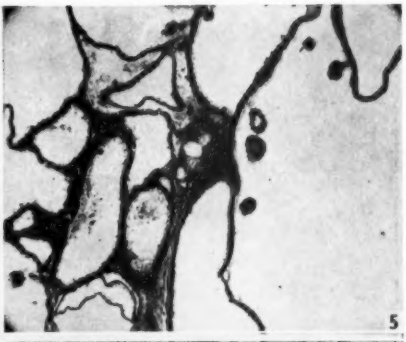

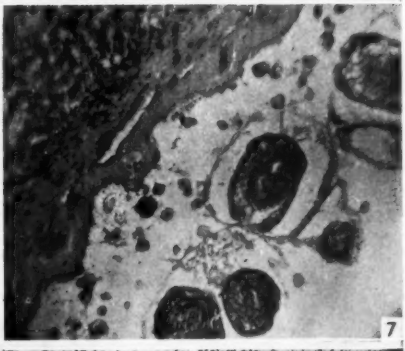

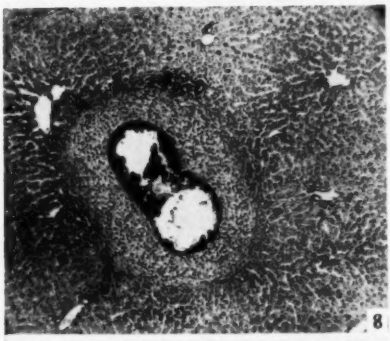

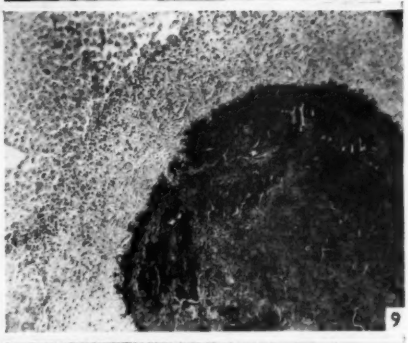

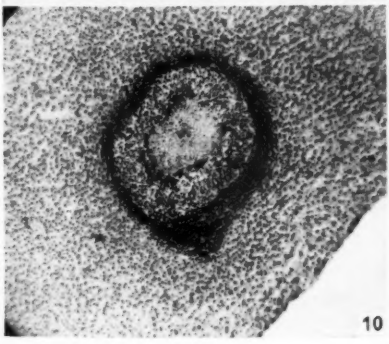

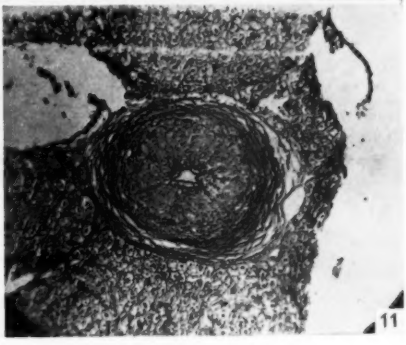

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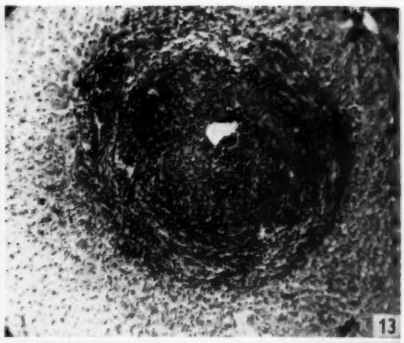
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EXPLANATION OF PLATES VII AND VIII

Plate VII. Sections of larval *Echinococcus sibiricensis* in livers of experimentally infected animals

Fig. 4. Common field vole, 14 days after experimental infection; cells of germinal membrane prominent. × 100. (All sections shown were stained with haematoxylin-eosin.)
Fig. 5. Common field vole, 45 days after experimental infection, showing early scolices. × 50.
Fig. 6. Common field vole, 5 months after experimental infection (see Text-fig. 3). × 100.
Fig. 7. Brown lemming, 5 months after experimental infection; subgerminal membrane strongly developed. × 100.
Fig. 8. Varying lemming, 20 days after experimental infection. × 50.
Fig. 9. Varying lemming, 5 months after experimental infection. × 50.
Fig. 10. Hamster, 10 days after experimental infection. × 50.
Fig. 11. Domestic rabbit, 30 days after experimental infection. × 50.

Plate VIII. Section of larval *Echinococcus sibiricensis* in livers

Fig. 12. Horse, 45 days after experimental infection. × 50.
Fig. 13. Rhesus monkey, 30 days after experimental infection. × 50.
Fig. 14. Rhesus monkey, 2½ months after experimental infection.
Fig. 15. Sterile larval vesicles of *E. sibiricensis* in liver of man; natural infection of unknown duration. × 50.
Fig. 16. Area in liver of man (see Fig. 15), showing unusual degree of proliferation of subgerminal layer of larval *E. sibiricensis*. × 50.

(*MS. received for publication* 9. VII. 1955.—Ed.)

CAMALLANIDAE PARASITIC IN SOME CEYLON FISH

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INTRODUCTION

One of us (C.H.F.), while studying the food habits of fresh-water fish, came across a number of nematodes belonging to the family Camallanidae (Nematoda: Spiruroidea). No record has been made so far of Camallanidae from Ceylon. This induced us to investigate their occurrence in some of the local fish.

MATERIALS AND METHODS

The fish used in this study were collected from ponds and paddy fields in Nugegoda, Marawila, Narawila, Bingiriya and from irrigation reservoirs in Kekirawa. Live fish were immersed immediately after netting in 15–25 % formalin estimated roughly under field conditions. The larger specimens were later injected with the same solution of formalin when brought to the laboratory. The guts were dissected out from the fish, teased out in Petri-dishes and examined with a dissecting microscope. The larger specimens were visible to the naked eye. The parasites were picked up with dissecting pins (when large) or with a pipette and preserved in 70 % alcohol. Fixation of the parasites was good under these conditions, but most of them were coiled, especially at the tail end. The morphology was studied using both 25 % glycerine in 70 % alcohol and beechwood creosote.

The species of fish investigated were *Puntius vittatus* Day, *P. chola* Ham. Buch., *P. filamentosus* Cuv. et Val., *P. dorsalis* Jerdon, *Rasbora daniconius* Ham. Buch. (Cyprinidae); *Nemacheilus botia botia* Ham. Buch. (Cobiitidae); *Clarias batrachus* Linnaeus (Clariidae); *Ophicephalus punctatus* Bloch, *O. striatus* Bloch (Ophiocephilidae); *Anabas testudineus* Bloch, *Macropodus cupanus* Bloch (Anabantoidae); *Etroplus maculatus* Bloch, *E. suratensis* Bloch (Cichlidae); and *Panchax panchax* Ham. Buch. (Cyprinodontidae).

RESULTS

Three species of Camallanidae falling within two genera were detected in the guts of the fish examined, the species being *Camallanus anabantis* Pearse, *C. sweeti* Moorthy and *Procamallanus planoratus* Kulkarni. Table 1 gives in a tabulated form the results of the examinations.

Descriptions of the parasites are not made, as they are readily available, but mention must be made of the variations observed in the anatomy of the three species. Chakravarty (1939) reported certain differences in the anatomy of *Camallanus anabantis* which were also seen in our specimens. In addition to these, one female has a rounded posterior end. In *C. sweeti*, a number of males and females were observed without the three spines (mucrones) at the tip of the tail,

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this ending in a point. The majority of these specimens were males, one of which had three small bosses at the tip of the tail where the spines (mucrones) should have been. The females of *Procamallanus planoratus* had two very inconspicuous bosses on the two sides of the rounded tail.

The rate of infection of both species of *Ophicephalus* with adult *Camallanus sweeti* was high, while that of the other two species of parasites in these hosts was low. The infection rate of *Anabas testudineus* with *Camallanus anabantis* was moderate, while with *C. sweeti* it was low. In *Clarias batrachus* the rate of infection with *Procamallanus planoratus* was high in certain localities. In the other species of fish found parasitized, the infection rate was low. Third stage larvae of *Camallanus sweeti* were observed, one in each host, in *Ophicephalus punctatus* and *Clarias batrachus*.

Table 1. *The distribution of parasites in the fish examined*

Species of fish	No. of specimens examined	Occurrence of parasites		
		C. anabantis	*C. sweeti*	*P. planoratus*
Puntius vittatus	259	0	0	0
P. chola	13	0	0	0
P. filamentosus	13	×	0	0
P. dorsalis	47	0	0	0
Rasbora daniconius	42	×	×	0
Nemacheilus botia botia	12	0	0	0
Clarias batrachus	26	0	×	×
Ophicephalus striatus	5	0	×	×
O. punctatus	19	×	×	×
Anabas testudineus	181	×	×	0
Macropodus cupanus	18	0	0	0
Etroplus maculatus	47	0	0	0
E. suratensis	4	0	0	0
Panchax panchax	15	0	0	0

× = positive. 0 = negative.

Camallanus anabantis

Pearse (1934) described *C. anabantis* from *Anabas testudineus* in Siam. In 1939, Chakravarty redescribed the same species from the same host in India. He pointed out the similarities and dissimilarities between his material and that of Pearse. The similarites observed were in the shape and size of the buccal capsule and the number and type of ridges on the valves of the buccal capsule. The dissimilarities were centred round the posterior portion of the specimens, the characters being the presence or absence and the number of spines at the tip of the tail in both sexes. The specimens examined by us show both types of characters described by Pearse (1934) and Chakravarty (1939). Pearse (1934) himself noted that the spines at the tip of the tail may be two or three. Laying importance on the buccal characters for specific diagnostic purposes as did Chakravarty (1939), these specimens are all considered as variations of the species *C. anabantis*. Pearse (1934) obtained a female of this species from *Clarias batrachus* in Siam. Three new host species, *Ophicephalus punctatus*, *Puntius filamentosus* and *Rasbora daniconius* are recorded for this species.

Camallanus sweeti

Moorthy (1937) described this species from *Ophicephalus gachua* Ham. Buch. from India. Although the majority of specimens collected by us resembled those described by Moorthy (1937), a few did not possess the spines (mucrones) usually associated with this species. Most of the specimens which showed this difference were males. As they resembled *Camallanus sweeti* in their buccal characters, these specimens are considered as variations of that species. In explaining the occurrence of the male with three bosses instead of the spines (mucrones), Prudhoe in a personal communication suggested that the spines may be retractile. If it is so, this provides an explanation for the observations of Pearse (1934) and Chakravarty (1939) on these structures of *C. anabantis* and our observations on the similar structures of *C. sweeti* which were obtained from four new hosts, *Anabas testudineus*, *Ophicephalus punctatus*, *O. striatus* and *Rasbora daniconius* from Ceylon. Moorthy (1938) records third stage larvae of *C. sweeti* from *Barbus puckelli* Ham. Buch., *Lepidocephalus thermalis* Cuv. et Val., *Puntius ticto* Ham. Buch. and *Gambusia* sp. which has been introduced to India. We have come across them in *Ophicephalus punctatus* and *Clarias batrachus* in Ceylon.

Procamallanus planoratus

This species was described by Kulkarni (1935) from the intestine of *Clarias batrachus* from India. The females of the specimens collected by us possess at the sides of the rounded tail two small bosses which are very inconspicuous and may not be readily observed except when looked at from a particular angle. Since our specimens agree with those of Kulkarni (1935) except for these bosses, which he does not mention, and as there seems to be considerable variation in the Camallanidae studied, we consider them as variations of *Procamallanus planoratus*. This species has been recorded from India in *Clarias batrachus* (Kulkarni, 1935) and now we have found it in the same host in Ceylon; *Ophicephalus punctatus* and *O. striatus*. *O. punctatus* and *O. striatus* are new hosts for this species. No Camallanidae were encountered in *Macropodus cupanus*, *Puntius chola*, *P. vittatus*, *P. dorsalis*, *Nemacheilus botia botia*, *Etroplus maculatus*, *E. suratensis* and *Panchax panchax*, although these specimens came from the same ponds where other infected fish were.

Life history

Briefly the life history is as follows: the adult female passes newly hatched larvae into the host's intestine, from which they reach the water with faeces. The larvae, when eaten by copepods, undergo further development and become third stage larvae, which encyst in the body cavity of the copepod (Li, 1935; Pereira, Dias & de Azevedo, 1936; Moorthy, 1938). Moorthy (1938) found third stage larvae naturally infecting *Barbus puckelli*, *Lepidocephalus thermalis*, *Puntius ticto* and *Gambusia* sp., the last-named introduced from Italy. On the evidence that advanced stages of *Camallanus sweeti* were found in *Barbus puckelli* and *Lepidocephalus thermalis*, he considered these two species as necessary second intermediate hosts. The definitive host in this case was a carnivorous fish

Ophicephalus gachua. Hyman (1951) considers the evidence for a second intermediate host inconclusive.

Among the fish infected with Camallanidae, *Clarias batrachus*, *Ophicephalus striatus*, *O. punctatus*, *Rasbora daniconius* and *Anabas testudineus* are carnivores of widely varying degree in the adult stages, although they feed extensively on copepods in the young stages (Mookerjee, 1945; Mookerjee, Ganguly & Bhattacharya, 1948; Mookerjee & Mazumdar, 1950; Fernando, in press). Therefore infection of these species may take place either at the adult level from infected 'carrier hosts' such as the species enumerated by Moorthy (1938) or at the young stage by the ingestion of infected copepods.

Puntius vittatus, which is the commonest prey of the carnivorous fish mentioned above and which is almost entirely an algal feeder, with very few copepods recorded from its intestine (Weerakoon & Fernando, 1952; Fernando, in press), was found to be free of camallanid infection in Ceylon. Other small fish which might be considered as food for these carnivores are *Macropodus cupanus*, *Panchax panchax*, *Nemacheilus botia botia*. These fish were not infected either, although they came from the same ponds as the infected carnivores. The larger carps, *Puntius chola* and *P. dorsalis*, were also free from infection. These may serve as food for the carnivores, especially in their young stages. It is worth noting that *P. filamentosus*, which was seen infected only once, was absent in the majority of habitats where camallanid infection was high. It is evident from this that infection, if it occurs by ingesting infected small fish, should be extremely low.

Species of small fish like *Macropodus cupanus*, *Panchax panchax*, *Nemacheilus botia botia*, *Puntius chola* and *P. filamentosus* feed on copepods (Chacko & Kuriyan, 1948; Fernando, in press). The species in which Moorthy (1938) found larvae of *Camallanus sweeti* feed on copepods. Perhaps conditions in the intestines in these species may be favourable for these parasites and hence some of them may have undergone further development as observed by us in *P. filamentosus*, and by Moorthy (1938) in *Barbus puckelli* and *Lepidocephalus thermalis*. Moorthy (1938) interpreted this as evidence for a second intermediate host.

Thus, considering the food habits of the hosts parasitized, it may be safely concluded that a second intermediate host is not required for infection of the carnivorous species of fish, infected smaller fish acting only as 'carrier hosts'.

Pereira *et al.* (1936) working on the biology of *Procamallanus cearensis* Pereira, Dias and de Azevedo, a parasite of *Astyanax bimaculatus vittatus*, found that the small fish *Curimatus elegans* Steindachner became infected by eating the infected *Diaptomus*. The development proceeds in this fish up to the third and possibly the fourth stage when the development is arrested. The fifth stage was found to occur only in the definitive host *Astyanax bimaculatus vittatus*. They considered the small fish *Curimatus elegans* as a 'carrier host'. There is no evidence to doubt a different course of infection in other species of Camallanidae.

SUMMARY

1. *Camallanus anabantis* is recorded from Ceylon in *Anabas testudineus*, *Ophicephalus punctatus*, *Puntius filamentosus* and *Rasbora daniconius*. *P. filamentosus*, *R. daniconius* and *O. punctatus* are new hosts for this species.

2. *Camallanus sweeti* is recorded from Ceylon in *Anabas testudineus*, *Ophicephalus punctatus*, *Ophicephalus striatus* and *Rasbora daniconius*. One third stage larva was observed in each of *O. punctatus* and *Clarias batrachus*. *O. punctatus*, *O. striatus* and *R. daniconius* are new hosts for this species.

3. *Procamallanus planoratus* is recorded in *Clarias batrachus*, *Ophicephalus punctatus* and *Ophicephalus striatus* from Ceylon. *O. punctatus* and *O. striatus* are new hosts for this species.

4. Variations in the anatomy of the posterior ends of the three species are mentioned.

5. No Camallanidae were obtained from *Macropodus cupanus*, *Puntius chola*, *P. dorsalis*, *P. vittatus*, *Panchax panchax*, *Nemacheilus botia botia*, *Etroplus maculatus* and *Etroplus suratensis*.

6. Reasons are given for suggesting direct infection of the host from the first intermediate host, although a 'carrier host' may exist.

We are indebted to Mr A. Indrasena, Superintendent, Freshwater Fisheries, Department of Fisheries, Ceylon, for assistance in obtaining and identifying some of the fish used in this study. We greatly appreciate and are thankful to Mr S. Prudhoe of the British Museum (Natural History) for the valuable criticisms and suggestions in the preparation of this paper.

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(*MS received for publication* 3. VIII. 1955.—Ed.)

THE SCOLEX OF *TETRABOTHRIUS AFFINIS* (LÖNNBERG), A CESTODE FROM *BALAENOPTERA MUSCULUS* L., THE BLUE WHALE

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(With 24 Figures in the Text)

The material used in the present study was part of a collection of parasitic worms, from whales in the Ross Sea, given to the writer by Mr W. Ross Cockrill, F.R.C.V.S. A general account of this collection has already been published (Rees, 1953).

In the collection were included twenty specimens of *Tetrabothrius affinis* from the intestine of four Blue Whales, *Balaenoptera musculus* L. Lönnberg (1892) first described this worm as *Diplobothrium affine* from *Balaenoptera borealis* Less. off the coast of Finmark, Norway. Later, Baylis (1926) recorded it from *B. musculus* from the south Shetlands and Cape Town, South Africa, and from *B. borealis* off Cape Point, South Africa. Baer (1932) recorded it from the same two hosts. In his recent revision of the family Tetrabothriidae Baer (1954) states that *Tetrabothrius wilsoni* (Leiper & Atkinson, 1914) Baylis, 1926 (*Oriana wilsoni* Leiper & Atkinson) is synonymous with *T. affinis*. This species was first reported from *Balaenoptera borealis* off the Bay of Islands, New Zealand, and a single specimen was found by the writer (1953) in *Physeter catodon* L. from the Ross Sea.

Baer (1954) has raised the family Tetrabothriidae to an order which he calls the Tetrabothridea. All the constituent species are parasites of five orders of sea birds and of Cetacea. According to Baer the Tetrabothridea of birds are more ancient than those of Cetacea and show a greater homogeneity in the structure of the scolex which is referred to as the 'classic' type of scolex for the order. In the Tetrabothridea of Cetacea there are, according to the same author, two lines of evolution. In *Tetrabothrius innominatus* Baer from *Steno bredanensis* Less. the scolex is of the 'classic' type resembling that of the species parasitic in birds. From this two types have developed, the first involves a hypertrophy of the apical region of the scolex as illustrated by *Tetrabothrius anophrys* (Baylis), *Strobilocephalus triangularis* (Diesing) and possibly *Priapocephalus* spp., and the second a modification of the structure of the bothridia involving, secondarily, an atrophy of the apical region, as seen in *Tetrabothrius affinis* (Lönn.), *T. ruudi* Nybelin, *T. forsteri* (Krefft) and *Trigonocotyle globiocephalae* Baer. In both lines the first sign of evolution is, apparently, an atrophy of the auricles.

Spätlich (1909) has made some fairly detailed descriptions of the anatomy of the scolex of the 'classic' type from bird hosts, e.g. *Tetrabothrius immerinus* (Abildg.) and *T. laccocephalus* Spätlich. None of the scolices of the 'developed' types which occur in Cetacea seems to have been described in detail. There is given below, therefore, a detailed account of the musculature, nervous and excretory systems of the scolex of *T. affinis*.

The specimens were fixed at the time of collecting in 5% formaldehyde. For the examination of the scolex series of transverse, sagittal and horizontal sections were cut at 10μ in thickness and stained with Heidenhain's Azan or Hansen's Trioxyhaematin.

EXTERNAL FEATURES OF SCOLEX

The scolex is large, quadrangular in shape with rounded corners. It measures 4 mm. in breadth and 2 mm. in length (Figs. 1, 2). The four large bothridia are hemispherical or sometimes almost spherical in shape, each occupying a quarter of the quadrant. The openings of the deep bothridial cavities are directed forwards, usually lying in the same horizontal plane with the edges rolled in to varying degrees. The forward direction of the bothridial openings is most clearly apparent in specimens fixed while still attached to the gut and removed subsequently. In shape, therefore, the scolex is different from the 'classic' type, which is longer than broad and in which the bothridial openings are widely open and face two dorsally and two ventrally. The bothridia are lined with cuticle continuous with that covering the scolex and when contracted the floor of the bothridium may be raised into irregular humps (Fig. 11). Each bothridium is provided with a lateral appendage, the auricle, which is reduced in size and somewhat fleshy. The auricles lie close together between the dorsal and ventral bothridia on each side; a flattened fleshy ridge passing across the apex of the scolex connects the auricles of one side with those of the other. This ridge is broadest in the centre and is lobed where it projects slightly over the central margins of the bothridia (Figs. 2, 9, 19). In this respect the scolex resembles that of *T. ruudi* Nybelin (1928) and differs from the 'classic' type where the auricles are more prominent.

Segmentation begins immediately behind the scolex. It is sometimes indistinct at first as the surface may be irregularly and deeply corrugated, making exact determination of the limits of the segments extremely difficult.

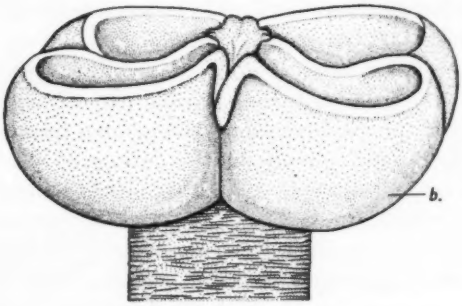
MUSCULATURE OF SCOLEX

The musculature of the scolex is derived, mainly, from the muscles of the strobila. To understand the former, therefore, it is necessary to examine the arrangement of the muscles immediately behind the scolex and to follow them forwards.

(*a*) *Superficial musculature.* The superficial musculature of the strobila consists of an outer layer of fine circular muscles followed by an inner layer of single longitudinal muscle fibres. These continue into the scolex, being present everywhere below the surface. At the apex of the scolex the superficial muscles form a network of diagonal fibres (Fig. 19).

(*b*) *Deep musculature.* Immediately behind the scolex the deep musculature consists of outer longitudinal and inner transverse muscles. The longitudinal muscles are arranged in a broad band of large muscle bundles composed each of a large number of fibres. Lönnberg (1892) described two layers of longitudinal muscle bundles in the strobila, but stated that in the neck region only the inner bundles are present. The fibres of neighbouring bundles show an anastomosis so that the number of fibres within a bundle does not remain constant. Some of the

bundles towards the outer side of the layer are smaller and may pursue a wavy course corresponding to the corrugations on the surface of the body. A fairly broad band of delicate transverse fibres lies within the longitudinal muscle layer. At the lateral margins some of these spread out and pass between the longitudinal muscle bundles to be inserted into the basement membrane, while others are continuous, laterally, passing around the outer side of the lateral nerve cords.



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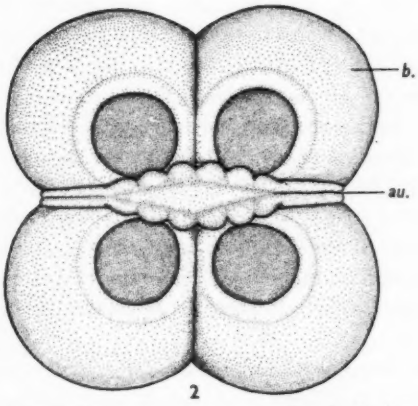

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Fig. 1. Scolex, lateral view.

Fig. 2. Scolex, anterior view.

Key to lettering of Figs. 1–24. (All drawings are semi-diagrammatic.) *a.b.n.* anterior bothridial nerve; *a.l.n.* antero-lateral nerve; *a.o.* apical organ; *au.* auricle; *b.* bothridium; *c.a.v.* central ascending branch of ventral vessels; *c.d.v.* central descending branch of ventral vessel; *c.g.* cerebral ganglion; *d.a.* anastomosis of dorsal excretory vessels; *d.b.n.* dorsal bothridial nerve; *d.d.m.* dorsal diagonal muscles; *d.m.* 1. diagonal muscles (series 1); *d.m.* 2. diagonal muscles (series 2); *d.m.* 3. diagonal muscles (series 3); *d.n.* dorsal nerve; *d.v.* dorsal excretory vessel; *d.v.m.* dorso-ventral muscles; *e.d.b.* excretory vessel of dorsal bothridium; *e.m.* equatorial muscles; *e.v.b.* excretory vessel of ventral bothridium; *l.g.* lateral ganglionic swelling on nerve hexagon; *l.l.m.* lateral longitudinal muscles; *l.n.* lateral nerve; *l.o.m.* lateral oblique muscles; *m.l.m.* median deep longitudinal muscles; *m.m.* meridional muscles; *n.h.* nerve hexagon; *p.b.n.* posterior bothridial nerve; *p.l.n.* postero-lateral nerve; *r.c.* ring commissure; *r.m.* radial muscles; *s.m.* superficial muscles; *t.c.* transverse commissure; *t.m.* transverse muscles; *v.a.* anastomosis of ventral excretory vessels; *v.b.n.* ventral bothridial nerve; *v.d.m.* ventral diagonal muscles; *v.n.* ventral nerve; *v.v.* ventral excretory vessel.

Radial and dorso-ventral muscles are also present. Fuhrmann (1899) and Spätlich (1909) have regarded the degree of development of the musculature in this group as a specific character, but as pointed out by Baer (1954), the number of individuals of any one species present in the host may influence the size of the worms and consequently the degree of development of the musculature.

The deep musculature becomes considerably altered on entering the scolex.

(1) *Longitudinal muscles.* Immediately below the bothridia the longitudinal muscle bundles in the mid-dorsal and mid-ventral regions become concentrated into a large mass rounded on the inner face and indented on the outer face (Fig. 3). Those which enter the scolex dorso-laterally and ventro-laterally become inserted into the posterior faces of the bothridia, forming part of the retractor system of these organs. Laterally the longitudinal muscle bundles remain small but distinct and form a continuous band passing on the outside of the lateral nerve cord (Figs. 3–5). There is no immediate division of the lateral bundles into a dorsal and ventral group, as described by Baer (1954) for *Tebrabothrius immerinus*, by Zilluff (1912) for *T. umbrella* and by Spätlich (1909) for *T. laccocephalus*. The early division of the lateral longitudinal muscles in these three species is probably associated with the greater development of the auricles. The median muscle masses extend forwards through the scolex dorsally and ventrally between the bothridia (Figs. 4–6, 11, 14, 15), becoming compressed as the available space narrows. Finally, each muscle mass divides into two groups of fibres one being applied to the adjacent faces of each of the two dorsal and two ventral bothridia (Figs. 6, 7). As these muscle masses pass forwards some of the fibres become inserted at successive levels into the inner surface of each bothridium, so that in a series of transverse sections passing up the scolex the number of fibres appears to decrease (Figs. 4–7). The last of the fibres are inserted into the bothridia a short distance behind the level of the anterior bothridial nerves. Contraction of these muscles would, presumably, assist in maintaining the position of the aperture of the bothridia in the same horizontal plane. Such powerful muscles are not present in the scolex of the 'classic' type and seem to be developed here in relation to the nature of the bothridia.

Laterally the small bundles of longitudinal muscles continue forwards in an unbroken line (Figs. 3–7), the fibres at the dorsal and ventral ends of the line being inserted, in series, into the walls of the bothridia as the latter approach their maximum diameter and consequently encroach upon the interbothridial area. Immediately below the auricles the lateral longitudinal muscle band divides on either side into a dorsal and ventral group the fibres becoming inserted into the auricles and the walls of the neighbouring bothridia (Fig. 8). This series of muscles forms a less powerful set of retractors than do the median longitudinal muscles, but presumably they assist in orientating the apertures of the bothridia and in altering the position of the auricles.

(2) *Transverse muscles.* Immediately behind the bothridia and in the neighbourhood of the formation of the dorsal and ventral muscle masses, the transverse muscles become more conspicuous than in the early part of the strobila. At the four 'corners' of the scolex most of the transverse muscles spread out into a broad

fan passing immediately below each bothridium towards the body wall (Fig. 3). A few of the fibres are continuous laterally. Passing forwards the transverse muscles disappear within a very short distance and thereafter are not present in the scolex.

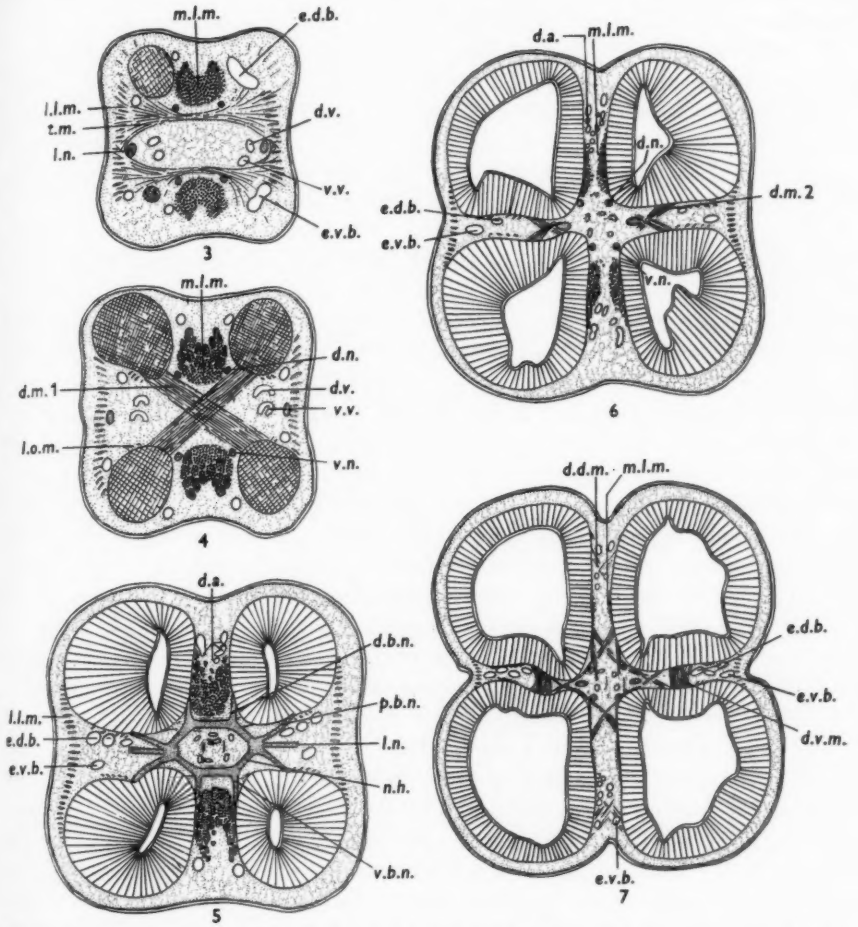


Fig. 3. Transverse section through base of scolex showing dorsal and ventral group of deep longitudinal muscles and excretory vessels passing under bothridia.

Fig. 4. Transverse section through scolex showing diagonal muscles (series 1).

Fig. 5. Transverse section through scolex showing nerve hexagon.

Fig. 6. Transverse section through scolex showing origin of diagonal muscles (series 2).

Fig. 7. Transverse section through scolex showing diagonal muscles (series 2) and band of dorso-ventral muscles between bothridia on either side.

(3) *Extrinsic muscles.* The musculature of the scolex is further complicated by the presence of powerful extrinsic muscles associated with the bothridia. These may be grouped under the headings (*a*) diagonal muscles, (*b*) lateral oblique

muscles and (*c*) dorso-ventral and transverse muscles. The latter are not a continuation of the transverse muscles of the strobila, but a new development. In transverse sections these systems of muscles form a very characteristic pattern where they intercross in the central region of the scolex, the arrangement being reminiscent of other species of *Tetrabothrius*.

(*a*) *Diagonal muscles.* Diagonal muscles are present in the central region of the scolex and also, more superficially, dorsally and ventrally.

Central diagonal muscles. There are three main series of central diagonal muscles which will be considered in sequence passing from the base of the scolex to the apex.

Series 1. Immediately in front of the transverse muscles mentioned previously are two broad bands of delicate diagonal muscle fibres crossing one another in the centre of the scolex (Fig. 4). These connect the bothridia which are diagonally opposite to one another. They are of brief extent and resemble, somewhat, those described by Zilluff (1912) from the same region in *T. umbrella*.

Series 2. A second series of diagonal muscles appears in the central region of the scolex a short distance in front of the nerve hexagon. These originate from the adjacent faces of the dorsal and ventral bothridia between the lateral nerve cords and the lateral excretory vessels (Fig. 6). These muscles are not only diagonal but pass obliquely forwards, so that their whole extent is not apparent in one transverse section. Shortly after their origin they cross one another before passing on either side of the lateral nerve cord and continue forwards across the scolex to the bothridium diagonally opposite. Mid-dorsally and mid-ventrally the fibres cross one another and become inserted into the adjacent faces of the two dorsal and two ventral bothridia near the central region of the scolex. This series of muscles, therefore, delimits a diamond-shaped area in the centre of the scolex in which are situated the nerves and central excretory vessels. The series continues from just in front of the nerve hexagon to the level of the entry of the anterior bothridial nerves into the bothridia (Figs. 6, 7, 16). In *T. laccocephalus* Spätlich (1909) figures a similar series of muscles but in the same horizontal plane.

Series 3. A third series of diagonal muscles appears in the region of the anterior nerve centre and continues from here to the anterior extremity of the scolex (Figs. 8, 9, 17, 18). The series is not completely isolated from series 2, as a few diagonal muscles are present in the intervening space. The arrangement of the muscles in this series follows the same general plan as in series 2 except that they tend to be more nearly in the same horizontal plane and to extend farther out laterally. The fibres originate from the muscular wall of the auricles, pass towards the centre of the scolex, crossing one another on either side about half-way along the level of the bothridia. They then continue, as do those of series 2, and are inserted into the wall of the bothridium diagonally opposite delimiting again a diamond-shaped area in the centre of the scolex (Figs. 8, 17). Passing farther forwards through the scolex, the dorsal and ventral lobes which overhang the bothridia begin to appear (Fig. 9). The diagonal muscles are then inserted into the muscular core of the lobe which is diagonally opposite, though a few may be inserted into that which is diametrically opposite. The points of origin of the muscle fibres gradually move in a little way from the lateral margin.

Dorsal and ventral diagonal muscles. A series of diagonal muscles is present dorsally and ventrally between the adjacent faces of the two dorsal and two ventral bothridia extending from not far below the level of the posterior loop of the ventral excretory vessel to the anterior extremity of the scolex (Figs. 7–9). These also, for the main part, take a forwardly oblique course and consist of relatively short fibres as the distance traversed by them is not great. Near the apex of the scolex some of the fibres pass directly across in one plane. Similar muscles are figured by Zilluff (1912) for *T. umbrella* and by Baer (1954) for *T. immerinus*, but are more delicate and more restricted in distribution.

(*b*) *Lateral oblique muscles.* The muscles of this series do not cross one another. They are delicate fibres which arise from the adjacent sides of the bothridia, laterally, over a fairly wide area, first appearing in the neighbourhood of the first series of diagonal muscles (Fig. 4). They continue to arise from the bothridia up to the level of the posterior loop of the excretory vessel (Figs. 4–7). They pass obliquely forwards and outwards to be inserted into the posterior face of the auricles of the same side (Fig. 8).

(*c*) *Dorso-ventral and transverse muscles.* A fairly powerful, isolated band of dorso-ventral muscles extends from the dorsal to the ventral bothridium on either side immediately in front of the origin of the second series of diagonal muscles (Fig. 7). Apart from these, dorso-ventral and transverse muscles occur sporadically in the central region of the scolex from the level of the nerve hexagon to the anterior extremity (Figs. 7–9, 14–18). They pass between the inner borders of the two dorsal and two ventral bothridia and, when they are all present simultaneously, demarcate a square or rectangular area in the centre of the scolex (Fig. 18). More posteriorly both these sets of fibres are rather delicate and sparsely distributed, but passing forwards they become more concentrated and better developed (Figs. 7, 16). The dorso-ventral muscles are most powerful and most extensive at the anterior extremity where they extend right across the median part of the scolex (Fig. 9). Together with the diagonal muscles they form an extensive muscular network in this region. Dorso-ventral and transverse muscles are present in other species of *Tetrabothrius* with some variations in detail, but the powerful network at the anterior end is not present in the 'classic' type of scolex.

The musculature of the scolex therefore is very complex, the large bothridia being provided each with a very efficient set of retractor and extrinsic muscles which control their movements most efficiently.

MUSCULATURE OF THE BOTHRIDIA AND AURICLES

The musculature of the bothridia is more complicated than that of the acetabula in the Cyclophyllidea. The cavity of each bothridium is lined by cuticle and the outer side delimited from the parenchyma of the scolex by a well-defined limiting layer. The main body of the bothridium is composed of bundles of radial muscles which form bands radiating through the wall (Fig. 10*a*, *b*). Some of the bundles which are adjacent to one another may cross instead of being strictly side by side, and there is a certain amount of anastomosis between neighbouring

bundles. The radial muscles are less well developed around the apertures of the bothridia. The other fibres constituting the musculature of the bothridia are the equatorial and meridional fibres, the arrangement of which can be well seen in sagittal sections. On the side next to the cavity of the bothridium the equatorial fibres are better developed on the part of the bothridia away from the centre of the scolex (Fig. 10*b*), where they are arranged in bundles of up to fifteen fibres interspersed between the radial muscles in the distal part of the bothridium. The bundles become gradually smaller, passing into the depth of the bothridium and finally disappear. On the opposite side of the bothridium, namely, that nearer the centre of the scolex (Fig. 10*a*), the equatorial muscles are not as extensive, consisting of single large fibres or of bundles of two or three fibres. These equatorial fibres are, of course, continuous around the inner side of the bothridium, being more powerful away from the central region. On the outer surface of the bothridium, that is, under the limiting layer, the opposite is the case. On the side near the centre of the scolex (Fig. 10*a*), the equatorial muscles consist of single very large fibres or bundles of two or three which continue from the apex of the bothridium into the depth moving inwards, slightly, between the radial muscles. On the outer side, away from the centre of the scolex (Fig. 10*b*), the equatorial muscles are less extensive. They consist here of large bundles of about fifteen fibres, but the bundles decrease rapidly in size, move inwards slightly and finally disappear. The combined action of the equatorial muscles, which are particularly well developed in the distal half of the bothridium, presumably regulates the size of the aperture and consequently the shape of the bothridium, altering it from a hemispherical to an almost spherical shape.

Meridional fibres are absent from the internal surface of the bothridium, but are well developed on the outer side below the limiting layer where they extend around the bothridium. Approaching the aperture, some of the meridional fibres pass inwards between the equatorial muscles and are eventually inserted around the aperture (Fig. 10*a*, *b*). Contraction of these muscles would enlarge the aperture and shorten the bothridium.

The muscles of the auricles are continuous with those of the bothridia (Figs. 8, 9). Apically they become separated at the lateral margins from the main muscles of the bothridia by a delicate partition. They consist of radial muscles and a few equatorial fibres.

NERVOUS SYSTEM

The nervous system consists of a nerve centre, a complicated system of nerves in the scolex and lateral, dorsal and ventral nerves in the strobila.

There are six longitudinal nerves running throughout the length of the strobila, a lateral nerve on either side and two dorsal and two ventral nerves. Lönnberg (1892) mentioned only the lateral nerves. The lateral nerves lie a short distance to the outer side of the lateral excretory vessels and just within the longitudinal muscles. The two dorsal and ventral nerves are situated about one-third of the distance from the lateral margin on either side and lie immediately outside the transverse muscle layer. They are smaller in diameter than the lateral nerves.

The dorsal and ventral nerves pass forwards into the scolex and come to lie on either side of the inner margins of the dorsal and ventral median longitudinal muscles (Figs. 3, 4). The lateral nerves on entering the scolex curve outwards

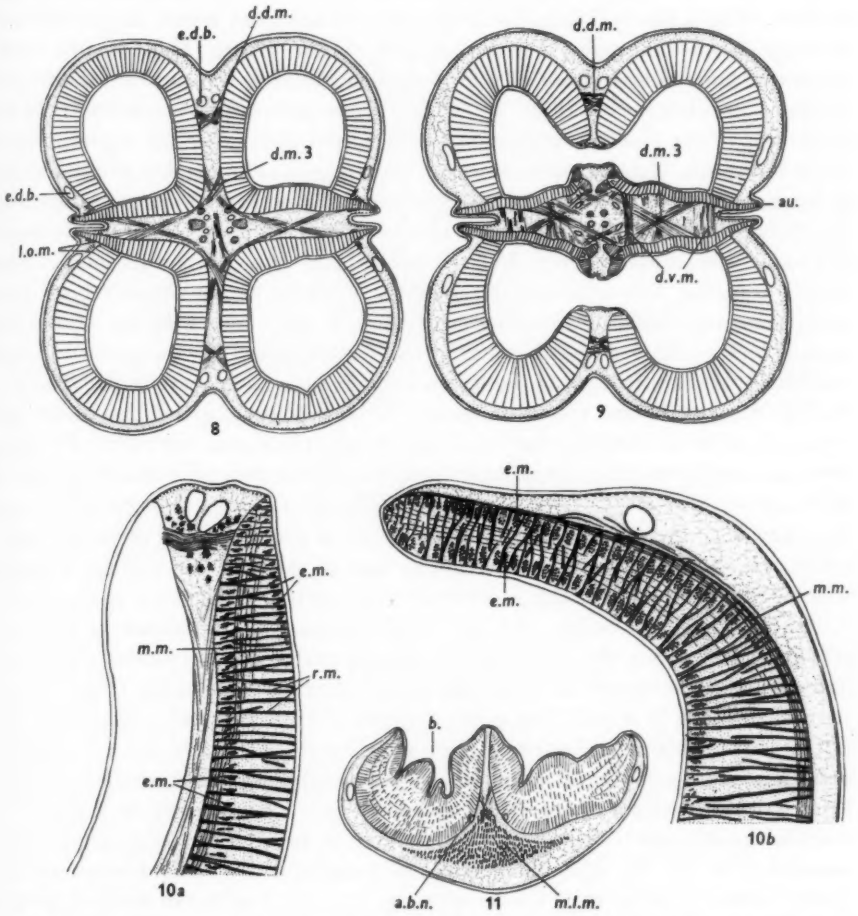


Fig. 8. Transverse section through scolex showing diagonal muscles (series 3) and dorsal and ventral diagonal muscles.

Fig. 9. Transverse section through apex of scolex showing auricles and muscles in this region.

Fig. 10*a*, *b*. Sagittal sections through (*a*) inner side and (*b*) outer side of wall of bothridium.

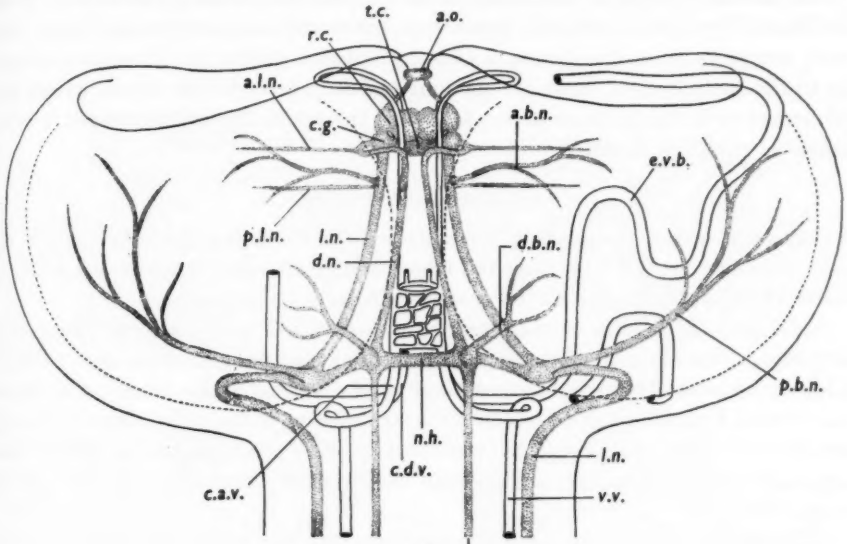
Fig. 11. Horizontal section near surface of scolex showing deep longitudinal muscles.

between the proximal regions of the dorsal and ventral bothridia on either side (Fig. 12). They then loop sharply round and continue for a short distance towards the centre of the scolex. At this point all six longitudinal nerves are connected with one another by a hexagonal nerve ring which will be referred to as the nerve hexagon (Figs. 5, 12, 14). Where the lateral nerves join the nerve hexagon there is

a ganglionic swelling. From this ganglion arises, on either side, a dorsal and a ventral posterior bothridial nerve (Figs. 5, 12, 14, 15). These diverge dorso-laterally and ventro-laterally on either side of the lateral nerve cord, taking a slightly forward course to enter the bothridia about half-way along their adjacent borders. Within the bothridia the nerves give off branches which can be followed for some distance through the muscular walls of these organs (Figs. 12, 15). From the dorsal and ventral angles of the nerve hexagon arise the two dorsal and two ventral bothridial nerves (Figs. 5, 12, 14). These pass dorsally and ventrally on either side of the median longitudinal muscles and soon enter the adjacent faces of the bothridia. They also branch within these organs (Fig. 12), but the branching is not as extensive as in the case of the posterior bothridial nerves. Lönnberg (1892) made no mention of dorsal and ventral nerves in the scolex nor of the dorsal and ventral bothridial nerves. In front of the nerve hexagon the six longitudinal nerves continue forwards, curving slightly inwards as the diameter of each bothridium approaches its maximum (Figs. 6, 7, 12). They then lie around the central zone of the scolex, two dorsally, two ventrally and one on either side, and continue almost to the apex of the scolex where lies the main nerve centre (Fig. 12). A short distance behind the nerve centre the lateral nerves give off on either side a pair of anterior bothridial nerves (Figs. 12, 16). These pass outwards, diverging from one another to enter the adjacent margins of their respective bothridia almost in the same plane as their point of origin. Within the bothridia they give off several branches (Fig. 12). Each bothridium, therefore, is provided with three bothridial nerves which branch fairly extensively so that all parts of the wall are supplied with nerves. Lönnberg (1892) mentioned only one bothridial nerve to each bothridium. Between the points of origin of the anterior bothridial nerves on either side there arises from the lateral nerve a pair of postero-lateral nerves which pass almost directly outwards to supply the lateral margins of the scolex (Figs. 12, 16). A short distance in front of the point of origin of these nerves on either side, the lateral nerves enlarge to form a cerebral ganglion (Figs. 12, 20). The two cerebral ganglia are connected with one another by a broad transverse commissure which runs across the centre of the scolex (Figs. 12, 17). Immediately in front of the cerebral ganglia and connected with them is a broad-lobed ring-shaped commissure (Figs. 12, 18). This consists of two dorsal and two ventral lobes and two lateral lobes on either side, the latter being connected with the cerebral ganglia immediately behind (Figs. 12, 20). The ring commissure partly overlaps the transverse commissure behind, and here the dorsal and ventral lobes are connected with one another by an X-shaped commissure which passes dorso-ventrally through an aperture on the anterior border of the transverse commissure (Fig. 18). This is reminiscent of the arrangement observed in the nervous system of *Taenia taeniaeformis* (Rees, 1951). From the anterior margin of the ring commissure there arises from each of the two lateral lobes on either side an anterior nerve (Figs. 9, 12, 20). The four anterior nerves continue forwards, turn towards the centre and terminate in the apical organ (Figs. 12, 19, 20).

The dorsal and ventral nerves passing up the scolex turn sharply outwards, near the transverse commissure, pass just beyond the lateral nerves and then turn in to

join the outer side of the cerebral ganglion on either side (Figs. 12, 17, 20). Just before each joins the ganglion it gives off an antero-lateral nerve which extends outwards to supply the antero-lateral regions of the scolex and the auricles. The



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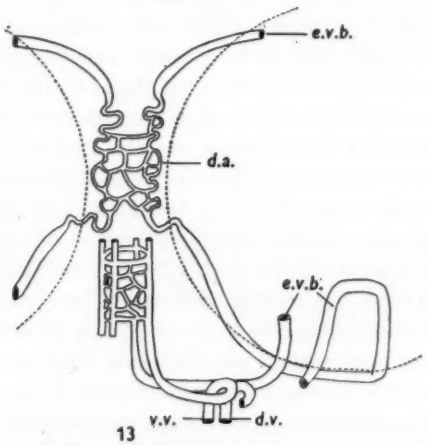

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Fig. 12. Reconstruction showing dorsal half of the nervous system and part of the course of the ventral excretory vessel on entering the scolex.

Fig. 13. Anastomosis of the excretory vessels of the dorsal bothridia and the anastomosis of the excretory vessels from the central zone.

only detail given by Lönnberg (1892) of the nervous system at the anterior end is that there is a commissure connecting the lateral nerves.

The apical organ of *Tetrabothrius affinis* (Figs. 12, 19, 20) may have a sensory function. It is an oval structure with a small central cavity, and is situated in

a shallow depression at the apex of the scolex. It is very similar to that described by Spätlich for *T. laccocephalus* and, as the four anterior nerves terminate in it, it seems most likely that it is a sense organ of some kind.

The nervous system of the scolex in the Tetrabothridea has received very little attention. Two nerve cords are figured in transverse sections in some cases. The most comprehensive description is that by Spätlich (1909) for *T. macrocephalus*. As in the present case, there are six longitudinal nerves in the scolex which are connected with one another at the base and the apex. There is, however, a considerable variation in detail.

EXCRETORY SYSTEM

A dorsal and a ventral excretory vessel extend the whole length of the strobila on each side. The ventral is slightly the larger in diameter and is connected with its fellow of the opposite side at the posterior end of each segment.

A complicated system of excretory vessels is present in the scolex. The dorsal and ventral vessels enter the scolex within which they join on either side to form a much convoluted loop. These two loops wind asymmetrically around the dorsal and ventral bothridia and extend into the central zone of the scolex. The excretory system is further elaborated by anastomoses where the vessels of either side approach one another mid-dorsally and mid-ventrally and again in the central zone.

If one of the ventral vessels is followed forwards into the scolex and its course traced, it will eventually emerge as the dorsal vessel. Due to the complicated arrangement it is simpler to follow the vessel in this direction. On entering the scolex the ventral vessel loops on itself and turns in a right-angle bend towards the ventral half of the central zone (Figs. 4, 12, 20). Near the centre it makes another right-angle turn, narrows in diameter and passes forwards as an ascending branch to the apex of the scolex. At the apex it turns outwards, forming a loop and returns as a descending excretory vessel through the central zone lying dorsal to the ascending vessel (Figs. 12, 20). There are, therefore, four excretory vessels in the central zone of the scolex, all derived directly from the ventral vessels as soon as the latter enter the scolex (Figs. 7–9, 10–18, 20). The position of these vessels relative to the nervous system can be seen in Fig. 12, and in some of the transverse sections (Figs. 5–9, 14–18). They lie within the area enclosed by the lateral, dorsal and ventral nerves which pass up through the scolex and pass through the nerve hexagon posteriorly (Figs. 5, 12, 14). Anteriorly the ascending and descending excretory vessels pass, respectively, ventrally and dorsally to the transverse and ring commissures and through the loop formed by the junction of the ventral and dorsal nerves with the cerebral ganglia (Figs. 12, 17, 18, 20). The lateral loop lies just behind the level of the apical organ. Immediately in front of the nerve hexagon the four vessels anastomose with one another for a short distance (Figs. 5, 6, 12–14, 20). On reaching the base of the scolex the descending excretory vessel which is now dorsally placed turns outwards in a right-angle bend almost parallel to the right-angle bend of the ascending vessel. It enlarges in diameter and continues laterally to a point about one-third of the

way along the adjacent borders of the dorsal and ventral bothridia (Figs. 12, 13, 20). Here it turns forwards and runs along the ventral face of the dorsal bothridium almost to the level of the origin of the anterior bothridial nerve. It then turns back upon itself, forming a more or less S-shaped bend directed laterally. This vessel will be referred to as the excretory vessel of the dorsal bothridium and will

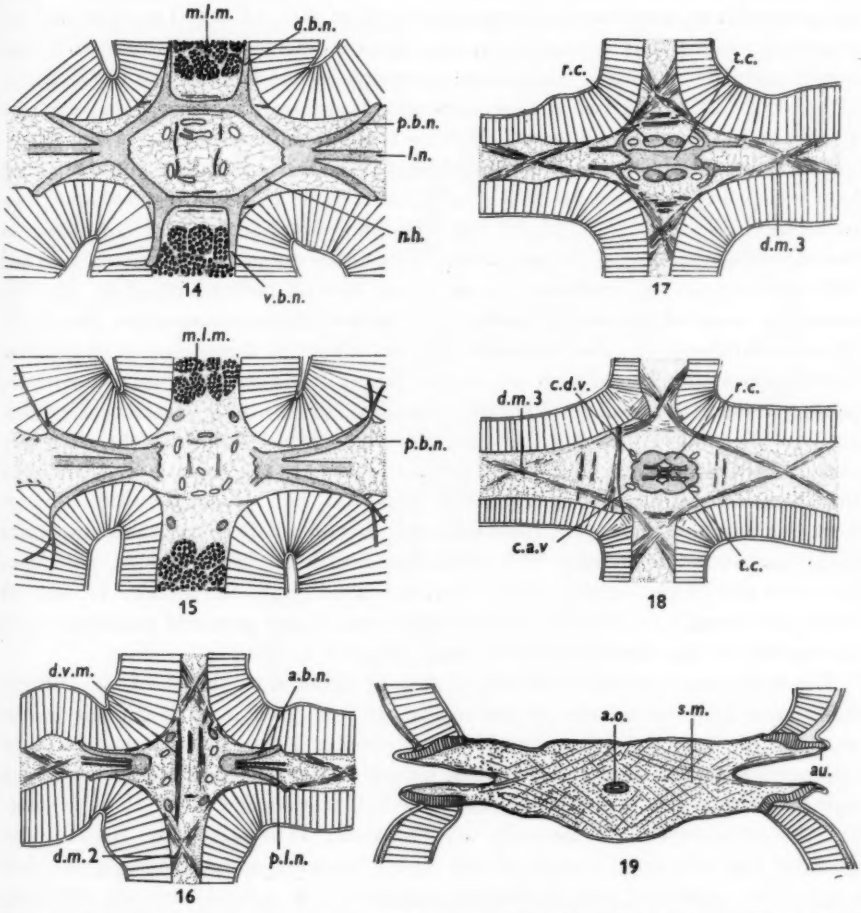

Fig. 14. Transverse section showing nerve hexagon and origin of posterior and dorsal and ventral bothridial nerves.

Fig. 15. Transverse section showing entry of posterior bothridial nerves into bothridia.

Fig. 16. Transverse section showing origin of anterior bothridial nerves and postero-lateral nerves.

Fig. 17. Transverse section through cerebral ganglia, transverse commissure and junction of dorsal and ventral nerves with the ganglia.

Fig. 18. Transverse section through ring commissure and X-shaped commissure.

Fig. 19. Transverse section through apex of scolex showing apical organ and network of superficial muscles.

be followed until it becomes continuous with the excretory vessel of the ventral bothridium and finally emerges from the scolex as the dorsal excretory vessel. The excretory vessel of the dorsal bothridium continues around the bothridium, passing obliquely forwards and around the margin to the mid-dorsal line (Figs. 8, 9, 12, 20). In the mid-dorsal line, between the two dorsal bothridia, the excretory vessels from both sides narrow, pass backwards close to one another for a short distance with an anastomosis between them (Figs. 5–7, 13, 20). The right and left excretory vessels then diverge from one another, increase in diameter and pass underneath the respective bothridium to emerge in the lateral zone of the scolex (Figs. 3, 13, 20). The two dorsal bothridia, therefore, are surrounded each by a more or less ring-shaped vessel. Having reached the lateral zone of the scolex, the excretory vessel of the dorsal bothridium turns forwards again to form a small loop which lies immediately behind and in the same plane as the S-shaped loop previously described (Figs. 12, 13, 20). The inner distal arm of the loop passes to the base of the scolex and crosses abruptly from the dorsal to the ventral side and will now become the excretory vessel of the ventral bothridium (Fig. 12). The excretory vessel of the ventral bothridium takes a simpler course than that of the dorsal bothridium. It turns forwards and runs along the dorsal face of the ventral bothridium without describing any loops (Fig. 12). It eventually turns outwards, passing around the apical region of the bothridium to the mid-ventral line (Figs. 6–9, 12). In the mid-ventral line between the two ventral bothridia the excretory vessels from either side narrow and behave in exactly the same way as those on the dorsal side, forming an anastomosis (Figs. 6, 7, 20). Eventually they diverge from one another, increase in diameter and pass obliquely under their respective bothridia to emerge laterally. The vessel then continues, on either side, passing to the outer side of the ventral vessel when the latter enters the scolex. It loops on itself just dorsally to the loop of the ventral vessel and proceeds posteriorly into the strobila as the dorsal excretory vessel (Figs. 4, 13, 20).

The dorsal and ventral excretory vessels of either side, therefore, are directly continuous with one another in the scolex. It is impossible to decide the precise point of junction of the dorsal and ventral vessels. If the arrangement had been symmetrical in the dorsal and ventral halves of the scolex, it would have been legitimate to assume that the junction came half-way along the length of the loop, but the arrangement is markedly asymmetrical. It is advisable, therefore, to consider the excretory vessels of the scolex without undue reference to their origin. The ascending and descending vessels in the central zone are obviously a direct continuation of the ventral vessel. It is interesting to note that the excretory vessel then passes first to the dorsal half of the scolex and then across to the ventral half, finally achieving the dorsal side again as it enters the strobila as the dorsal excretory vessel. This seems to be a most unusual arrangement, and as far as the writer is aware has not been found to exist in any other cestode in which the excretory system has been described. The only connexion between the excretory vessels of the right and left sides, in the scolex, is the slight anastomosis which occurs in the mid-dorsal and mid-ventral lines and the anastomosis between the four central vessels.

Spätlich (1909) has described the arrangement of the excretory vessels in the scolex of *T. laccocephalus*, but it appears to be very much simpler than in the present case. *T. laccocephalus* also has four excretory vessels in the central region of the scolex with an anastomosis between them, but the descending vessel on emerging from the central zone describes a few simple loops between the bothridia on each side and then descends to the strobila. The arrangement is symmetrical and there is no crossing of the vessels from the dorsal to the ventral side of the scolex. Due to the position of the bothridial openings, the excretory vessels cannot surround the bothridia, so none appears mid-dorsally and mid-ventrally.

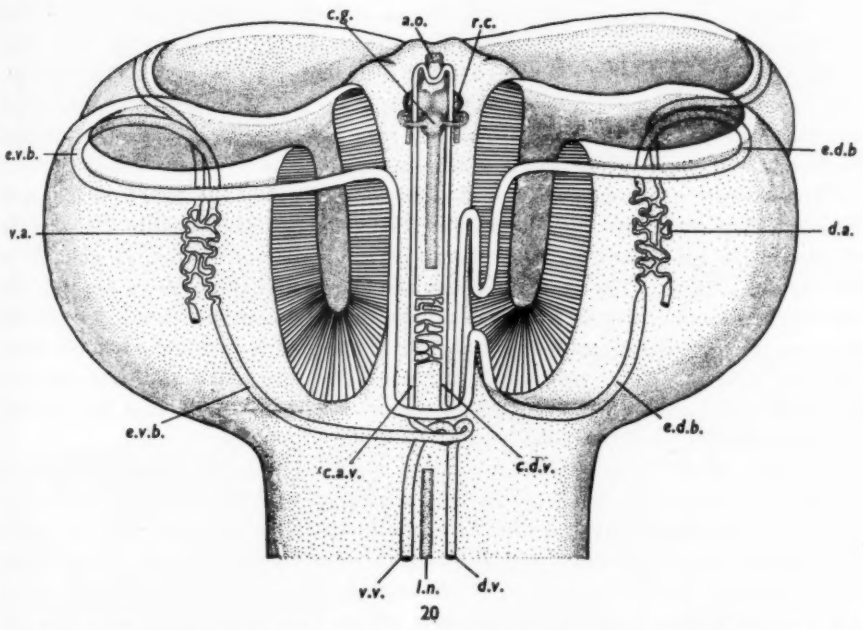

Fig. 20. Reconstruction of the left half of the excretory system of scolex from the left side with part of the bothridia removed.

The differences in the internal anatomy of the scolex of *T. affinis* as compared with the 'classic' type, can all be related to the unusual shape of the scolex. The almost spherical bothridia with their forwardly directed apertures are much more powerful than is normally the case. This necessitates a better and more extensive development of both the intrinsic and extrinsic bothridial muscles. The nervous system, likewise, is more elaborate as nerves must supply the highly developed muscles. The arrangement of the excretory vessels is quite unusual, extending to all parts of the scolex, forming, presumably, an efficient excretory mechanism.

THE EFFECT OF *TETRABOTHRIUS AFFINIS* ON THE INTESTINE OF THE HOST

Some of the specimens given to the writer had been fixed while still attached to the host's intestine, small pieces of the intestine being cut out around the attached worms. Some of these were sectioned with the worms still *in situ*, while in others the worms were removed and the portion of the intestine to which they had been attached was sectioned separately.

The whole thickness of the intestinal wall was not available, but only the mucosa consisting of the villus layer, the crypt layer and the muscularis mucosae. It is, however, unlikely that the submucosa and the muscular and serous coats would be involved in any changes resulting from the attachment of the bothridia to the mucosa. The material was fixed in 5 % formaldehyde at the time of collection. The fixation of the mucosa is not sufficiently good for a detailed examination of the histology, but even with this relatively poor fixation changes in structure can be seen at the points where the bothridia are attached.

The structure of the mucosa is quite typical. Villi cover the entire surface and between their bases are the openings of the glands of Leiberkühn. The glands extend to the muscularis mucosae, being perpendicular or irregularly oblique to the surface (Fig. 22). The spaces between the glands and the cores of the villi are filled by the lamina propria which contains reticular and other fibres. A reticular framework is present around the blind ends of the glands, forming a narrow layer between them and the muscularis mucosae. The muscularis mucosae runs in a straight line parallel to the surface of the mucosa and consists of a fairly thick layer of transverse muscle cells followed by longitudinal muscle cells.

On removal of the worm, after fixation, four small swellings were apparent where the mucosa had been drawn into the cavities of the bothridia (Fig. 21). A vertical section through two of these swellings is illustrated in Fig. 22, while Figs. 23 and 24 are vertical sections through a worm still attached to the mucosa. It will be seen that the mucosa is drawn into the cavities of the bothridia, all of which face directly forwards in the same plane. The 'plug' of mucosa fills the cavity and may be constricted around its base where the margin of the bothridium contracts like a sphincter to ensure a firm hold. The muscularis mucosae, which normally runs in a straight line, is also pulled outwards, slightly, below the mucosa 'plugs'.

Quite definite structural changes are apparent in the mucosa contained within the cavity of each bothridium. There is no trace of villi on the surface (Fig. 21). These being very delicate have apparently broken down. In Fig. 21 their absence from the surface of the 'plugs' can be clearly seen in comparison with their presence on the surrounding mucosa. In some cases the destruction of the surface proceeds more deeply so that the distal ends of the glands of Leiberkühn may be damaged. Their normal apertures are then no longer apparent, instead, they open widely the cells of their walls spilling out on to the surface (Fig. 23). In places the broken down distal parts of the glands and the remains of the villi form a layer of protoplasm with nuclei over the surface (Fig. 24). The most striking change is the

pronounced increase of fibres in the lamina propria. These form a thick layer over much of the surface of the 'plug' and around its base. They also form a dense network between the glands of Leiberkühn (Figs. 22–24). It is probable that the

Fig. 21. Four swellings on the surface of the mucosa, where the bothridia were attached, showing absence of villi on the swellings and presence of villi on the surrounding mucosa. (Photomicrographs by J. E. Welborne.)

Fig. 22. Vertical section through two mucosa 'plugs' showing increase in fibres in the lamina propria and other histological changes.

Figs. 23, 24. Vertical sections through the scolex of a worm attached to the mucosa showing the mucosa 'plug' in the cavity of the bothridium and histological changes in the mucosa.

worm would need to be attached for a considerable time before these changes take place. It is possible, also, that the worm does not change its position once it is attached. If it did so the damage to the part of the mucosa concerned would appear to be permanent, and a heavy infestation, where the worms changed their position frequently, would result in a considerable reduction of the functional area of the mucosa.

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(*MS. received for publication* 4. VIII. 1955.—Ed.)

LIFE HISTORY OF *GIGANTOBILHARZIA HURONENSIS* NAJIM, 1950. A DERMATITIS-PRODUCING BIRD BLOOD-FLUKE (TREMATODA-SCHISTOSOMATIDAE)*

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(With 19 Figures in the Text)

INTRODUCTION

The presence of a new species of bird schistosome in Ann Arbor, Michigan, was suspected when a new type of schistosome cercaria was found emerging from *Physa gyrina*. These cercariae appeared very similar to the cercariae of *Schistosomatium douthitti* but experimentally failed to infect white mice (*Mus musculus* L.). It was decided to investigate this matter by working out the life cycle of this schistosome in the laboratory, studying the morphology and some biological aspects of each stage; and by obtaining specimens of the local avifauna and examining them for adult worms. The results of this investigation are set forth below.

HISTORICAL NOTES

Rudolphi in 1819 described the first bird schistosome, a Brazilian species from *Sterna* sp. which he named *Distomum canaliculatum*, later changed to *Ornithobilharzia canaliculatum* (Rudolphi, 1819) Odhner, 1912. The genus *Gigantobilharzia* was erected in 1910 by Odhner for the reception of a new species of bird schistosome—*G. acotylea*—which he discovered in *Larus fuscus* in Sweden. Szidat (1930) added a second species to the genus *Gigantobilharzia*, describing *G. monocotylea* n.sp. from *Anas bosches* in east Prussia. Lal (1937) in India obtained a single male specimen of a new schistosome from the cattle egret (*Bulbulcus ibis coromandus*) which he placed in the genus *Gigantobilharzia* as *G. egreta* n.sp. Mehra (1940) raised the genus *Gigantobilharzia* to subfamily rank, a change which has not been accepted by other workers. Brackett (1942) described five new avian schistosomes from the United States, one of which was a species of *Gigantobilharzia*, namely, *G. lawayi* n.sp. He also completed experimentally the life cycle of *Cercaria gyrauli* Brackett, 1940, which proved to be the cercarial stage of another species of *Gigantobilharzia* which was named *G. gyrauli* n.comb., thus becoming the first to elucidate the life cycle of a species within the genus.

The new species of avian schistosome described below, *G. huronensis* n.sp. from the goldfinch, was reported in abstract by Najim (1950). Finally, Tanabe (1951) designated *Cercaria sturniae* Tanabe as *G. sturniae* Tanabe, 1948, n.comb.

* Contribution from the Department of Zoology, University of Michigan.

MATERIALS AND METHODS

Materials. Most of the snails used as intermediate hosts during this study were raised in the laboratory. About 1000 snails were collected in the field. The snail was identified as *Physa gyrina* (Say). Most of the snails were collected from a back-water pool of the Huron River at Delhi, near Ann Arbor, Michigan.

The experimental hosts that were found to be susceptible were chicks and canaries. White mice (*Mus musculus* L.), domestic ducklings and a mourning dove (*Zenaidura macoura*) failed to become infected. Natural final hosts were found by shooting birds, mostly from the same location where the intermediate host was found.

The eggs of the parasite were obtained from faeces of infected canaries. Mother sporocysts and daughter sporocysts were studied almost exclusively from laboratory-infected snails. The cercariae were studied from laboratory-infected snails as well as from natural infections. The adult worms were obtained mostly from the mesenteric veins around the intestines of the infected birds. A few worms, mostly immature, were recovered from the lungs and the livers of some birds in the early stages of the infection. Usually it is difficult to obtain whole specimens, but in the course of this work one young male and four females were recovered entire. One more female was obtained almost complete. Nearly a hundred slides of whole mounts of both sexes were prepared. The anterior part of ten males and twelve females were sectioned, some transversely and others longitudinally. Living specimens were also studied.

Experimental infections. Young laboratory-raised snails about 2–4 weeks old were exposed to miracidia recently hatched from the eggs. One of the following two methods was used:

(1) The faeces of infected canaries were collected on wet filter-paper on the bottom of the cage. These faeces were ground with sand, then placed in a 1 l. distilling flask with a vertical side arm. The mass was diluted with river or spring water until the water filled the neck and the arm of the flask. An envelope of black photographic paper covered the whole flask, excluding the side arm which remained uncovered. This arm was exposed to light by placing the flask with its contents close to an electric light. Within several minutes the hatched miracidia were usually observed in the side arm, showing their phototropic nature. These miracidia were separated into groups of from four to six miracidia in 10 ml. stender dishes. A young snail was then introduced into each dish and the container filled with spring or river water and left from 4 hr. to over-night under an electric light. This same technique was also followed when using intestines and livers of infected birds instead of the faeces.

(2) The eggs in the faeces of the final host hatched without mechanical help by placing the faeces in a 75 ml. stender dish containing spring or river water. The eggs hatched but required more time than the first method. The miracidia were either separated into smaller containers, as in the above-mentioned method, or a number of young snails were placed in the dish containing faeces and left covered for a few hours. This rough method of exposure was followed when the

purpose of exposing was to keep a stock of infected snails in the laboratory. The percentage of infection in the laboratory was 68·5 % (taken from 274 surviving snails) and the percentage in nature during the summer was 5 % (taken from 256 surviving snails).

Exposed snails were kept in culture dishes in an aquarium room. These containers were of 2500 ml. capacity or larger and were half filled with dechlorinated tap water. In each dish seven to fourteen snails were usually placed. The snails were fed soaked, dried maple leaves. A small amount of calcium carbonate on the tip of a small spatula was added to each dish every 6–7 days. Later this food was replaced by preserved strained spinach. A small amount of spinach, on the tip of a small spatula, was added to each culture dish with half the amount of calcium carbonate. At the end of every 2 days the water was changed, and new amounts of spinach and calcium carbonate were added. This food was found to be convenient, since it is always available, does not require much space for storing, and needs no preparation, whereas dry maple leaves take about 1 month of soaking and several water changes before they are ready to use. Also when using spinach one can avoid losing small snails, which are usually hidden between the maple leaves and may be thrown away when the leaves are changed.

Chicks and canaries were infected by exposing them repeatedly to a heavy suspension of cercariae. A total of about 550–1400 cercariae were used for each bird. Smaller numbers of cercariae were usually used for canaries and larger numbers for chicks. The author was able to infect one canary by exposing it only once to about 550 cercariae for 20 min. The feathers, if present, were removed from the abdomen and the bird was placed in infested water in 500 or 1000 c.c. beakers. The beakers were covered and placed in a warm water bath. The infested water in the beaker was allowed to come in contact with the abdomen of the exposed bird. The exposure usually was for 20–45 min.

In nature, one male goldfinch (*Spinus tristis tristis*) was found to be infected bisexually. A male cardinal (*Richmondena cardinalis*) was found infected unisexually and only one female was recovered from the small veins around the intestine. No eggs were found in the intestinal wall or liver. The seminal receptacle of the female worm was empty.

Eggs were found in great numbers in the lumen and mucous layer of the intestine of the goldfinch, and it was possible to complete the life cycle from these eggs by infecting the snail host, canaries and chicks.

Technique. Adult worms were usually obtained from the small veins around the intestine of the infected birds from a region shortly posterior to the duodenum to the pyloric caeca. Occasionally worms were recovered from the pyloric region of chicks. Fine needles were used for recovering the worms. The blood vessels, containing the worms, were dissected carefully in a spot close to the middle of the body of the worm and the needle was inserted carefully in the loop made by the freed part of the body of the worm. The worm was then pulled out gently. The technique was more successful when the worm was in a larger vein. The recovered worms curled up outside the host blood. Neither cooling in the refrigerator nor the use of menthol were successful in relaxing the worms. At about the time of death

the worms were relaxed. The adult worms were fixed in hot Bouin or hot alcohol, formalin and acetic acid, and the paraffin embedding technique was followed for sectioning. Delafield's haematoxylin, Mayer's paracarmine and Mayer's HCl carmine were the stains used for the whole mounts. Mayer's HCl carmine and Heidenhain's haematoxylin were the stains used for the sectioned specimens. Eosin, fast green and indulin were the counterstains used. Graded ethyl alcohol and xylol were used for dehydration and clearing. However, terpineol was found to be useful as a clearing agent, since the body of the worm is fragile, and cannot stand too much hardening caused by xylol. Measurements of the whole worms were taken by drawing the outlines of the worm with the aid of a microprojector. By using a universal map-measuring instrument the length was calculated.

Unfixed eggs were studied in 0·940% saline solution, physiological saline solution and in spring water. The latter was used for studying hatching activities. The location of the eggs in the intestinal tissue was studied from section of those tissues.

The miracidium was studied in living condition without stains or with vital stains. These were neutral red and nile blue sulphate. It was possible in this way to study most of the internal structures. Silver nitrate (La Rue's unpublished technique with modifications) was useful for studying the epidermal plates, excretory pore, bristle patches and papillae. Carnoy's was used for fixing and affixing the miracidia on the slides. Boiling 10% formalin was used for fixing specimens to be measured. Delafield's haematoxylin, Mayer's HCl carmine and Mallory's triple stain were used for staining permanent mounts of the miracidia. Miracidia also were studied both living and in serial sections after penetration into the snail tissue.

Mother and daughter sporocysts were studied living, in permanent mounts and in sections. Neutral red and nile blue sulphate were used for living specimens. Delafield's haematoxylin and Mayer's HCl carmine were used for the permanent mounts.

Cercariae were studied, living, in dechlorinated tap water, for the excretory system. Saturated aqueous alizarin, neutral red and nile blue sulphate were the vital stains used. Permanent mounts were made of cercariae shed from the snails and of those dissected from the snails, and were stained with Mayer's HCl carmine, Harris's haematoxylin or Delafield's haematoxylin. The haematoxylins were also used for staining sections of the cercariae within the snails. Cercariae were measured after fixation in boiling 10% formalin.

The total number of wild birds examined was eighteen. Two species belonging to the family Fringillidae were found to be infected.

THE ADULT

Adult worms of *Gigantobilharzia huronensis* are elongate, filamentous and with fragile bodies. Males and females are almost alike in diameter and the females are longer than the males. Oral and ventral suckers are absent from both sexes, and very small papillae are present on the anterior end of both males and females

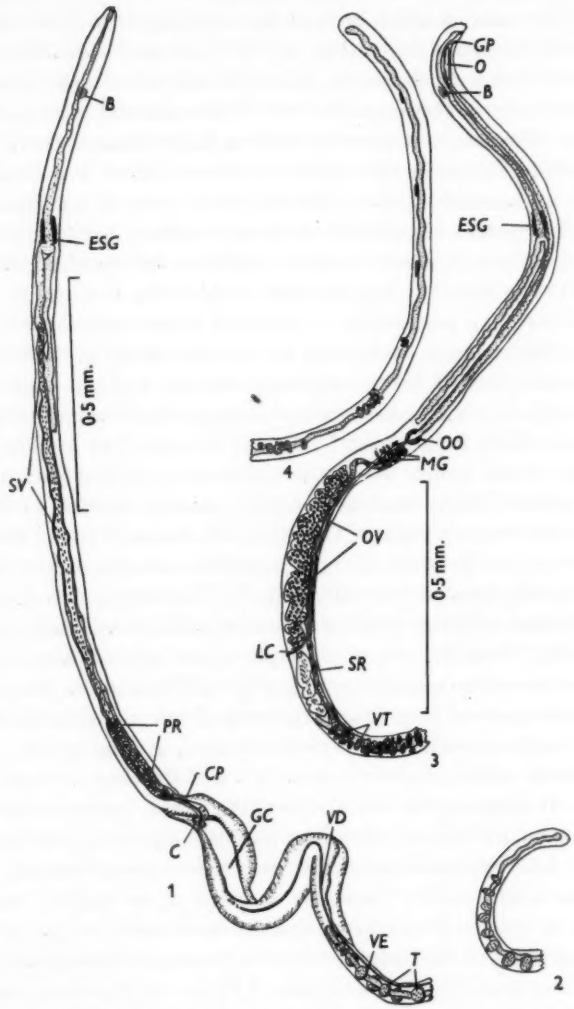
(Figs. 1, 3). Adult worms were never seen in copula. Both sexes were usually found scattered along the small intestine, in the small veins. Eggs were found in the faeces of the definitive host as early as 31 days after the first exposure. However, two sexually matured worms, male and female, were found 26 and 27 days after the first exposure, but they might not have reached their maximum length.

Specimens of the adult worms were catalogued as follows: U.S.N.M. Helm. Coll. No. 37334, paratypes, male fragment and a female, from chicks.

The male. The thread-like male is attenuated anteriorly and ends bluntly posteriorly. A short gynaecophoric canal is present (Fig. 1). No lateral expansions were observed at the posterior end (Fig. 2). The length of one young male (26 days old) is about 9·6 mm. The following measurements were taken from the anterior part of eight males recovered from intestinal veins of chicks; all the worms were fixed in hot Bouin. The width of the body anterior to the gynaecophoric canal, in the region of the intestinal caeca, averaged 51μ, ranging from 42 to 54μ. The width of the body posterior to the gynaecophoric canal averaged 40μ, ranging from 34 to 47μ. The length of the canal averaged about 1084μ, ranging from about 795 to 1550μ and the width averaged 70μ, ranging from 54 to 82μ. The canal is short compared to the length of the body of the male but is, more or less, well formed and defined, and muscular in structure. The cuticle is devoid of spines. The brain is located about 188μ from the anterior end, on the dorsal side of the worm pushing the oesophagus into a small curve in this region. The brain is usually seen as a lightly stained mass of very fine fibres (Fig. 1). Neurons were occasionally observed among the body tissue, darkly stained in sectioned specimens. The digestive system consists of a mouth, oesophagus, short intestinal caeca and a long common intestine. The mouth is subterminal and opens into a shallow buccal cavity which extends to the anterior end of the body (Fig. 5). The oesophagus averaged 478μ in length ranging from 375 to 690μ, and is weakly muscular in structure. It attaches to the intestinal caeca through a sinus-like connexion, made of the union of the intestinal caeca. Oesophageal glands surround the oesophagus for a short distance anterior to the junction of the oesophagus with the intestine (Fig. 1). They usually take more stain than the surrounding tissues. The intestinal caeca bifurcate for a short distance. The intestinal bifurcation of two worms recovered from chicks measured 170 and 289μ. The intestinal bifurcation of two worms from the intestinal veins of the canary measured 143 and 323μ, and one from the goldfinch was 374μ. The large range in measurements, even among specimens from the same host, may be due to difference in the age of the worms. The intestine after the short bifurcation reunites and extends as a single common intestine following a zigzag course and ends a short distance from the posterior end of the body (Fig. 2). The excretory pore is terminal posteriorly. The reproductive system of the male consists of a large number of testes, vasa efferentia, vas deferens, seminal vesicle, prostate, ejaculatory muscle, ejaculatory duct, cirrus pouch and a spiny cirrus (Figs. 1, 6). The testes are rounded to broadly oval in shape when fully developed, usually set close together in almost a lineal pattern, alternating with the wavy intestine. The testes are numerous in number (300± in a young male) and start shortly anterior to the posterior end of the

gynaecophoric canal and extend posteriorly to a point near the posterior end (Figs. 1, 2). The greater diameter of twenty-five testes averaged 35μ. Each testis is connected dorsally to a fine vas efferens which passes around the posterior of the testis and connects with the single vas deferens (Fig. 1). The vas deferens is usually located ventrally in the region of the testes and is about 4·5μ in diameter. It extends anteriorly, passing dorsal to the seminal vesicle, to a point about 607μ from the anterior end of the worm, where it turns posteriorly as a dilated seminal vesicle. The seminal vesicle is a large, elongated, sac-like structure, extending posteriorly in a looping manner. It is about 750μ in length and is made of two parts, a short thin-walled portion and a long thick-walled posterior part. The seminal vesicle is weakly muscular. The prostatic part averaged about 170μ in length and was made of glandular cells surrounding the lumen of the sperm duct. The prostate glands are surrounded, and in direct contact with a thin membrane which is differentiated at the beginning of the prostate region. This membrane constitutes the cirrus pouch, extending posteriorly, and covers the ejaculatory muscle and the ejaculatory duct. There is a space between the cirrus pouch and the ejaculatory duct itself with nuclei of an undetermined function (Fig. 6). At the posterior end of the prostate is the ejaculatory muscle. This muscle is well developed and was seen in almost every specimen. Its greater diameter averaged 22μ and surrounds the sperm duct. The ejaculatory duct is a rather thick-walled canal, extending to the genital pore and ending in a large spiny cirrus (Fig. 6). The cirrus is usually situated on the left side at the beginning of the gynaecophoric canal. Sperms were observed coming out through the cirrus in great number in living specimens. This might be caused by the pressure of the cover slide.

The female. Three entire females were obtained from chicken. Fixed in hot Bouin, they measured 16, 18 and 24 mm. in length. The worms were about 27, 30 and 46 days old, respectively. A fourth female, about 3 months old, almost complete, obtained from a canary, measured 29 mm. The following measurements were taken from fourteen females recovered from chickens' intestinal veins and fixed in hot Bouin. The width of the body anterior to the ootype averaged 51μ, ranging from 37 to 64μ. The body usually enlarges in diameter suddenly in the region of the ootype and stays large in the region of the ovary where it measured an average of 67·5μ, ranging from 57 to 78μ. The diameter of the body becomes smaller posterior to the seminal receptacle, averaging 51μ and ranging from 44 to 68μ. The body width near the posterior end averaged 37μ, and not much variation was found. A slight enlargement in the posterior end of the body was occasionally observed (Fig. 4). This enlargement was believed to be caused by the fixation, since the living worm was observed to be flexible at this end and continuous contractions and extensions were usually taking place. The brain is 172μ from the anterior end and is not easily seen in the females because of the presence of the uterus, in addition to the oesophagus. The digestive system consists of a mouth, oesophagus, intestinal caeca and a long common intestine. The mouth, also subterminal, opens into a funnel-like cavity, but unlike the male, no anterior extension of the buccal cavity was observed. The oesophagus, like that of the male, is weakly



Figs. 1–4. *Gigantobilharzia huronensis.* All the drawings were made from whole mounts.

Fig. 1. Anterior part of an adult male including gynaecophoric canal and a short portion of the testes region.

Fig. 2. Posterior end of an adult male.

Fig. 3. Anterior end of an adult female.

Fig. 4. Posterior end of an adult female.

Explanation of Figs. 1–19. All the drawings were made with the aid of a camera lucida. *Abbreviations: B*, brain; *BP*, bristle patch; *C*, cirrus; *CP*, cirrus pouch; *EJM*, ejaculatory muscle; *ESG*, oesophageal glands; *EXP*, excretory pore; *GC*, gynaecophoric canal; *GP*, genital pore; *LC*, Laurer's canal; *MG*, Mehlis's gland; *O*, ovum; *OO*, ootype; *OV*, ovary; *OVD*, oviduct; *PR*, prostate; *SR*, seminal receptacle; *SV*, seminal vesicle; *T*, testis; *VD*, vas deferens; *VE*, vas efferens; *VT*, vitellaria; *VTD*, vitelline duct.

muscular and averaged 549μ in length, ranging from 379 to 750μ. The oesophageal glands occupy the same position around the oesophagus as in the male, near the bifurcation of the intestinal caeca (Fig. 3). The intestinal caeca bifurcate as in the male, but, in contrast with the latter, the intestinal caeca in the female are longer, extending posteriorly to the posterior end of the seminal receptacle where they reunite (Fig. 3). The common intestine ends a short distance from the body end. One case of rebifurcation in the region of the vitellaria was observed. This is believed to be an anomalous case. The excretory pore is terminal and median. The reproductive system consists of an ovary, oviduct, ootype, Mehlis's glands, uterus, seminal receptacle, Laurer's canal, vitellaria and vitelline duct. The ovary, located about 1230μ from the anterior end of the body, is spiral in shape (Fig. 3) and extends about 431μ posteriorly. Connected to the posterior end of the ovary is the oviduct. This extends posteriorly for a short distance where it joins a large seminal receptacle, then it turns anteriorly making a sharp angle. The oviduct extends anteriorly to a short distance posterior to the rear end of the ovary and then turns back where it is joined by Laurer's canal. The oviduct through this passage makes several loops. From the point of connexion with Laurer's canal (Fig. 7), the oviduct turns anteriorly again, passing ventral to the ovary and extends anteriorly to a point about 112μ from the anterior end of the ovary where it becomes the ootype. Between the ootype and the anterior end of the ovary is the Mehlis gland, mostly located ventrally (Fig. 3). The ootype is a distinct structure with large epithelial cells surrounding its lumen and, occasionally, one ovum may be seen within it. From the ootype a simple uterus extends anteriorly and opens about 20μ posterior to the mouth opening (Fig. 3). The seminal receptacle is a large simple sac, often observed looped and, in bisexual infections, is filled with sperms. The extended seminal receptacle is spindle-shaped, averaging 136μ in length and 38μ at its greatest width. Laurer's canal is a well-developed organ in the female of this species. It opens on the dorsal side of the worm, between the ovary and the seminal receptacle, and has a protruding papilla bulging out through an atrium-like structure. Laurer's canal is narrow near the external opening, and enlarges as it continues inside until it becomes as large as or slightly larger than the oviduct which it joins. Numbers of sperms were seen in Laurer's canal. The vitellaria occupy most of the body posterior to the seminal receptacle. The vitelline follicles are arranged around the intestines. A single vitelline duct passes anteriorly and ends shortly before the ootype. The connexion between the vitelline duct and the oviduct was not observed. Up to seven eggs at a time were seen in the uterus.

THE EGG

Mature viable eggs of *G. huronensis* are spherical to very broadly ovoid in shape, without spines or indentation, straw coloured and with well-developed miracidia as seen through the shell. The vitelline membrane is not easy to see unless the egg swells in water; in this case the vitelline membrane appears to surround the embryo, leaving the granular material outside, between the vitelline membrane and the egg shell.

Measurements of forty viable mature eggs passed from a laboratory-infected canary and placed in 0·940 % saline solution were as follows: the length averaged 93μ, ranging from 86 to 105μ; the width averaged 88μ, ranging from 82·5 to 90μ. Seventeen eggs of the forty were spherical in shape. As many as seven eggs at a time can be seen in the uterus of a female from laboratory-infected hosts. The eggs of *G. huronensis* can be differentiated from other schistosome eggs by the size and by the fact that they are almost spherical, and without spines.

Examination of the host intestine, in serial sections and smears, has shown that the eggs are located in the mucosa and the submucosa of the intestine. The eggs can be seen crowded together, scattered, or in linear rows and varying in size and development. Eggs were seen rupturing the intestinal villi and ready to escape into the lumen of the intestine.

Hatching. Hatching of the eggs of *G. huronensis* agrees, generally, with what has been described for the other schistosomes. The matured viable eggs swell when placed in water and the narrow space between the embryo and the shell becomes larger; a translucent fluid fills the newly formed cavity between the embryo and the vitelline membrane. The miracidium becomes active, the anterior cilia start beating and the apical papilla contracts and extends. Shortly afterwards the rest of the cilia start beating and the miracidium becomes very active. At least three major movements are usually observed: (1) rotating around the periphery of the egg, (2) twisting and inverting the body, (3) boring against the shell and, sometimes, spinning the rest of the body during the accomplishment of this movement. While all these activities are going on, suddenly the egg shell breaks open and the miracidium is set free, moving around immediately and actively. When the miracidium is forced out under pressure it is usually sluggish and not very active.

To determine the time which is needed for hatching, a few drops of the faeces of a laboratory-infected canary were placed in a 75 ml. stender dish containing spring water. The eggs in this water were watched under a dissecting microscope until the first miracidium was observed swimming. The time of three observations averaged about 20 min. from the time the faeces was placed in water until the appearance of the first miracidium. However, in one case, using a side-arm distilling flask, one miracidium was observed swimming in the side arm in 11·5 min.

THE MIRACIDIUM

Form, size and covering. The miracidium of *G. huronensis* is pyriform in shape with a peculiar 'waist' around the middle of the body which is devoid of cilia (Figs. 9, 10).

Fifty miracidia from eggs passed by a laboratory-infected canary were relaxed in the ice-box for half an hour and fixed in boiling 10 % formalin. They measured as follows: the length averaged 158μ, ranging from 140 to 180μ; the greatest width averaged 58μ, ranging from 48 to 68μ.

Specimens of the miracidium were catalogued as follows: U.S.N.M. Helm. Coll. No. 37336, miracidium.

The body covering of the miracidium consists of twenty-two ciliated epidermal plates. These plates can be seen clearly and with definite boundaries in specimens treated with silver nitrate (Fig. 11). The distribution of the epidermal plates is: six plates in the first row, nine in the second row, four in the third row and three in the fourth row. There is a characteristic gap or 'waist' between the second and the third row, a region not covered with ciliated epidermal plates (Fig. 11). There is a notch in the middle of the base of each of the triangular-shaped plates of the first row. These notches usually surround a small circle about 1·5μ in diameter. One case of partial fusion of two neighbouring epidermal plates has been observed in the second row.

The cilia of the first row of plates, as is the case in other schistosome miracidia, start short, near the apical papilla, elongate close to the lateral papillae and become posteriorly as much as four times longer than those near the apical papilla.

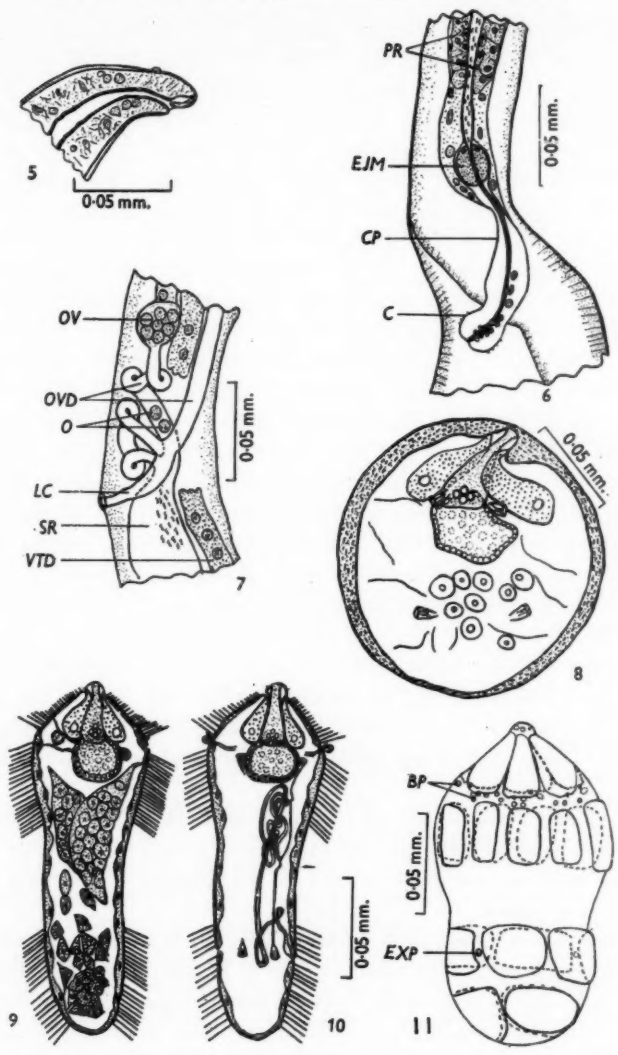
The fate of the ciliated epidermal plates was determined by dissecting and sectioning a number of exposed snails after intervals of from 30 to 60 min. Examinations of such material showed that these plates are retained by the miracidia after penetrating the tissues of the snail host, and will be lost inside the snail tissue, starting, usually, with the posterior ciliated plates.

Beneath the epidermal layer is a continuous subepithelial layer with ovoidal-shaped nuclei. Reisinger (1923) observed in *Schistosomum haematobium* a similar arrangement and shape of nuclei, but he called this layer the nucleus-bearing part of the epithelial cells, which is actually the subepithelial layer. Ozaki (1951) observed a similar structure of this layer in the miracidium of *Paramphistomum explanatum* (Creplin).

The primitive gut. The so-called primitive gut is a sac filled with granular material and extends from the tip of the apical papilla posteriorly to the anterior part of the brain. The primitive gut opens through the perforated dome-shaped tip of the apical papilla. There are four nuclei, usually grouped together, in the wall of the primitive gut. Observed in side view the gut appears to consist of two lobes. Mallory's triple stain was found to be selective for the nuclei of the gut. Nile blue sulphate also is useful for this purpose in the living miracidium.

In an effort to determine the function of the gut, approximately a hundred snails were exposed to about a dozen miracidia each for intervals of 30, 45 and 75 min. The snails were then transferred to a clean container and dissected with fine needles, and the miracidia recovered were stained with neutral red and nile blue sulphate. Some of these miracidia were in poor condition, some still retained the gut after penetration, while others had lost it completely. These cases were characteristic for each time group. This experiment showed that the primitive gut of this species is retained in some cases after penetration and may probably be used as an accessory penetration gland within the snail tissues.

The penetration glands. On each side of the primitive gut is a large unicellular granulated gland. Each gland opens anteriorly on the side of the primitive gut and extends to the brain posteriorly. They are used by the miracidium during penetration and are not seen in penetrated miracidia.



Figs. 5–11. *Gigantobilharzia huronensis*

Fig. 5. Longitudinal section of the anterior end of a male showing the anterior extension of the buccal cavity.

Fig. 6. End part of male 'endapparat', highly magnified.

Fig. 7. Connexions of the ovary, seminal receptacle and Laurer's canal; constructed from several specimens.

Fig. 8. Matured viable egg from the faeces of a canary.

Fig. 9. Miracidium showing the primitive gut, penetration glands, brain, triangular bodies and germ cells.

Fig. 10. A constructed miracidium showing one side of the excretory system, bristles in the anterior papilla, the lateral papillae and the lateral processes.

Fig. 11. Miracidium treated with silver nitrate showing the epidermal plates.

The lateral processes and the lateral papillae. The lateral processes are balloon-shaped structures, two on each side of the miracidium in this species (Fig. 10), located between the first and second row of the ciliated epidermal plates. No opening to the outside, nor any secretions were observed from these structures. A duct was observed in a few specimens leading inward from each process. Each duct terminates in a slightly enlarged vesicle which rests on the anterior part of the brain. A similar duct was observed in *Schistosomatium douthitti* by Price (1931). The observations of Sewell (1922), Tang (1938) and Wall (1941*a*, *b*) are in general agreement with this. However, in the miracidium of *Gigantobilharzia huronensis* there are two lateral processes on each side instead of one. Anterior to the lateral processes on each side is a papilla about 3μ in length.

The brain. The brain is located in the anterior third of the body. It is broadly ovoidal and may appear bilobed. Cells were observed over its surface and they usually stain deeply. Two lateral, forked nerve fibres have been noticed extending to the sides of the miracidium. On each side of the brain, apparently attached to the lateral nerves, is a large cell (Fig. 9). These cells are thought to be nerve cells.

Bristle patches. Bristle patches were observed in the living specimens attached to the periphery of small circles (3–4μ in diameter). The circles are situated between the first and second row of the ciliated epidermal plates, and can be seen clearly in specimens treated with silver nitrate (Fig. 11). No definite arrangement could be noticed for these circles, but they were mostly arranged in threes, twos, or singly. A few circular areas similar to those of the bristle patches described above were also observed in the apical papilla (Fig. 11). In the living miracidia, as well as in permanent mounts, short bristles were observed on each side of the apical papilla. These bristles are subterminal and can be seen only on the sides (Fig. 10).

Structures of undetermined nature. Very small circles (1·5μ in diameter) were observed in the apical papilla (Fig. 11). These circles are few in number, and almost the same size as those usually observed in the notches at the base of the first ciliated epidermal plates.

The excretory system. Two pairs of flame cells, an anterior pair and a posterior pair (Fig. 10), are present. The anterior pair is located at the sides of the brain, one cell on each side. The posterior pair is usually found in the vicinity of the third row of epidermal plates. The anterior and the posterior tubules from the flame cells join the convoluted collecting ducts near the middle of the body. Each collecting duct, after complicated loopings, discharges through the excretory pores on each side between the cells of the third row of epidermal plates (Fig. 11).

The germ cells. The germ cells are found in the posterior part of the body of the miracidium (Fig. 9), extending sometimes to the anterior half of the body. The so-called 'fibre-like extensions' can be seen extending from the cell body of some cells to the body wall of the miracidium. These fibre-like extensions are usually seen in cells which are isolated from the rest of the group.

The longevity of the miracidia. A simple experiment was carried out in the laboratory to show the longevity of the miracidia of this species. The temperature was about 70° F., and unnecessary light was avoided during the night. Thirty

miracidia were separated among eleven 10 ml. stender dishes. The miracidia were obtained by placing faeces containing eggs in spring water in a 75 ml. stender dish, and left to hatch. The water was checked for the hatched miracidia every 10 min. after the first miracidium hatched. The miracidia were recovered by decanting the water of the main dish to another dish and replacing it with fresh water and examining the decanted water for miracidia. Checking the living miracidia continued for 24 hr.; at intervals of 1 hr. for the first 14 hr. with an interval of 2 hr. between 9 and 11 hr. After 14 hr. the checking was made every 2 hr. until the end of the twenty-fourth hour. Part of the water had been replaced twice after 2 and 9 hr. After 24 hr. only five miracidia were left. Three miracidia appeared active while two showed very little activity. A young snail host was placed in the dish with these active miracidia and watched for 30 min. The miracidia moved actively, hit the snail several times, came into contact with the foot and head regions but no attempt to penetrate was observed. Under the conditions mentioned above, the mortality, after 24 hr., was about 83%. Even the active miracidia which had survived after this time were unable to continue the life cycle of the parasite. The disagreement in results reported by investigators who considered the duration of life of the schistosome miracidia, even in the same species, is due probably to the differences in methods and conditions under which each experiment was carried out. The result of this experiment is in close agreement with that of Short (1950) on the miracidium of *Schistosomatium douthitti* which was studied in the same laboratory.

The penetration of the miracidium. The actual penetration of the miracidium into the snail host was observed, and at the same time attention was paid to see whether or not there is any attraction between the miracidia and the snail host.

The miracidia anchor on the soft part of the snail and, as soon as they become firmly attached, they start boring. If about half of the body of the miracidium gets in, the rest will follow within a few seconds. Unsuccessful attempts were also observed and some miracidia failed to penetrate. Some became exhausted and were not able to re-attack. The miracidia were observed to attack almost any exposed soft part of the snail. The actual penetration was much easier to observe on the anterior part of the snail and the elongated foot. Very rarely were the tentacles attacked by the miracidia. The average time of ten cases of penetrations into ten snails of different sizes (2–6 mm. long) was 150 sec. with the extremes ranging from 90 to 210 sec. There was no confirmation of the idea of attraction between miracidia and the snail hosts, and it seemed that the miracidia meet their snail host by chance.

THE MOTHER SPOROCYST

The shape of the mother sporocyst of *Gigantobilharzia huronensis* does not differ from that described for other species of schistosomes, all of which have a comparatively simple, elongate, sac-like body. The anterior end is attenuated, the posterior is blunt (Fig. 12). The wall of the developed mother sporocyst is composed of a very thin cuticle and stellate cells. These cells have a rather large nucleus and conspicuous nucleolus (Fig. 13). Protoplasmic extensions can be seen across the lumen.

The majority of the mother sporocysts are located in the mantle near its edge. Occasionally they are found around the anterior part of the oesophagus. One case was recorded where a mother sporocyst was recovered from the tissues of the foot 8 days after exposure.

Mother sporocysts of the same age varied in size and development as did even those recovered from the same snail. Specimens from 13 to 15 days old usually showed almost all the stages of development from single germ cells to the sausage-shaped, immature daughter sporocysts. The immature daughter sporocysts were usually predominant in such matured mother sporocysts, and only a small amount of undeveloped germinal material was found scattered among the immature daughter sporocysts. The latter are found free in the lumen of the mother sporocyst. The living mother sporocysts do not show much activity and usually stick to the bottom of the container. The only detectable movement was a weak contraction of the anterior end of the young mother sporocysts. In the matured mother sporocyst it was even harder to detect such movement, and the only activity seen was among the immature daughter sporocysts.

The longevity of the mother sporocysts was not determined, but usually daughter sporocysts could be found in the liver of the snail after 14–15 days following exposure at laboratory temperature (60° F.). The shortest time (13 days) was recorded during a spell of warm weather. The period of maturation of the mother sporocyst seems to be the shortest compared to other schistosomes.

A birth pore was observed in a mature mother sporocyst 15 days old. An active well-differentiated daughter sporocyst was observed near the anterior end making its way out. A canal leading from the birth pore to this embryo was clearly seen. Cort & Olivier (1943) reported the presence of a birth pore in the mother sporocysts of *Trichobilharzia stagnicolae* (Talbot, 1936) McMullen & Beaver, 1945. Cort, Ameel & Olivier (1944) also reported a birth pore in the mother sporocysts of *Schistosomatium douthitti*.

Measurements of the mother sporocysts were made from permanent mounts at different stages of development. All the specimens were taken from laboratory-infected snails and stained with Mayer's HCl carmine. They measured as follows: two mother sporocysts (10 days old) fixed in hot alcohol, formalin and acetic acid were 1·218 and 1·562 mm. in length. Their width was 0·068 and 0·105 mm. A third mother sporocyst (13 days old) fixed in hot Bouin measured 1·245 mm. in length and 0·060 mm. in width. A fourth mother sporocyst (15 days old), fixative not recorded, measured 1·387 mm in length and 0·071 mm. in width.

These measurements are not conclusive, since the number of specimens examined was so limited. These figures are given to present in a general way the size of these mother sporocysts, which were obtainable entire and without damage.

THE DAUGHTER SPOROCYST

The daughter sporocysts make their appearance in the lumen of the matured mother sporocyst around the thirteenth day after exposure. They are elongate, sausage-shaped bodies, blunted posteriorly, while the anterior is attenuated and

covered with spines (Fig. 14). The daughter sporocysts are found usually in the liver 14–15 days after exposure. In an 18-day-old daughter sporocyst one may notice immature cercariae. The body wall of a 22-day-old daughter sporocyst (Fig. 15) is covered with a thin layer of cuticle. Two kinds of nuclei were differentiated: small ovoid nuclei differing in size and a second kind with more or less rounded nuclei and a visible nucleolus. When the daughter sporocyst contains well-developed cercariae the body wall becomes thin and the small ovoid nuclei of the body wall are predominant. These usually were close to the interior edge of the wall. All the above-mentioned daughter sporocysts were taken from snails infected during the summer months.

The shortest time for cercariae to leave laboratory-infected snails was 24 days after exposure during the summer time and 30 or more days during the winter (Najim, 1950). However, it was possible to shorten the period of time during the winter season by placing the snails near a heater after exposure. The snails were exposed to a 60 W. electric light 2–3 hr. a day during cloudy days. By this method cercariae would leave the snail within 24 days in January. In one case three snails were exposed in May and did not liberate cercariae until July when they were crushed and examined. All were found to be infected, and well-developed cercariae were seen in the daughter sporocysts.

A well-developed birth pore (Fig. 16) can be seen in mature daughter sporocysts. The anterior end including the birth pore is usually active and shows continuous contractions and movement in the living specimens. Eight daughter sporocysts (15 days after exposure) were recovered from the liver of a snail. They were fixed in hot Bouin's, stained with Mayer's HCl carmine and fast green, and averaged 0·500 mm. in length, ranging from 0·238 to 0·930 mm. The width averaged 0·038 mm., ranging from 0·030 to 0·051 mm. The longest daughter sporocyst showed large germ balls.

The oldest daughter sporocyst recovered complete was 18 days old (Fig. 14). It was fixed in hot Bouin and stained with Mayer's HCl carmine, and measured 1·500 mm. in length and 0·050 mm. in width.

THE CERCARIA

The cercaria of *Gigantobilharzia huronensis* is an apharyngeal furcocercous, brevifurcate distome with pigmented eyespots (Fig. 17). The following measurements were made on fifty cercariae from laboratory-infected snails, fixed in a 10% solution of boiling formalin. The body length averaged 240μ, ranging from 195 to 270μ; the body width averaged 72·5μ, ranging from 63·7 to 105μ; the tail stem length averaged 268·5μ, ranging from 255 to 300μ; the tail stem width near the body averaged 30μ, ranging from 22·5 to 45μ; the furca length averaged 146·8μ, ranging from 102·5 to 172·5μ; the furca width averaged 15μ, with not much range; the head organ width averaged 49·5μ, ranging from 45 to 60μ; the diameter of the acetabulum averaged 28μ, ranging from 22·5 to 30μ; the distance between the acetabulum and the anterior end of the body averaged 150μ, ranging from 120 to 180μ.

The tail is mucular with muscle cells similar to those in the cercaria of *Schistosomatium douthitti* as described by Price (1931). The furcae have fin folds. The excretory canal projects beyond the main body of the furca terminating in a slightly extended thimble-like structure (Fig. 17).

A specimen of the cercaria was catalogued as follows: U.S.N.M. Helm. Coll. No. 37335, cercaria, from *Physa gyrina* (Say).

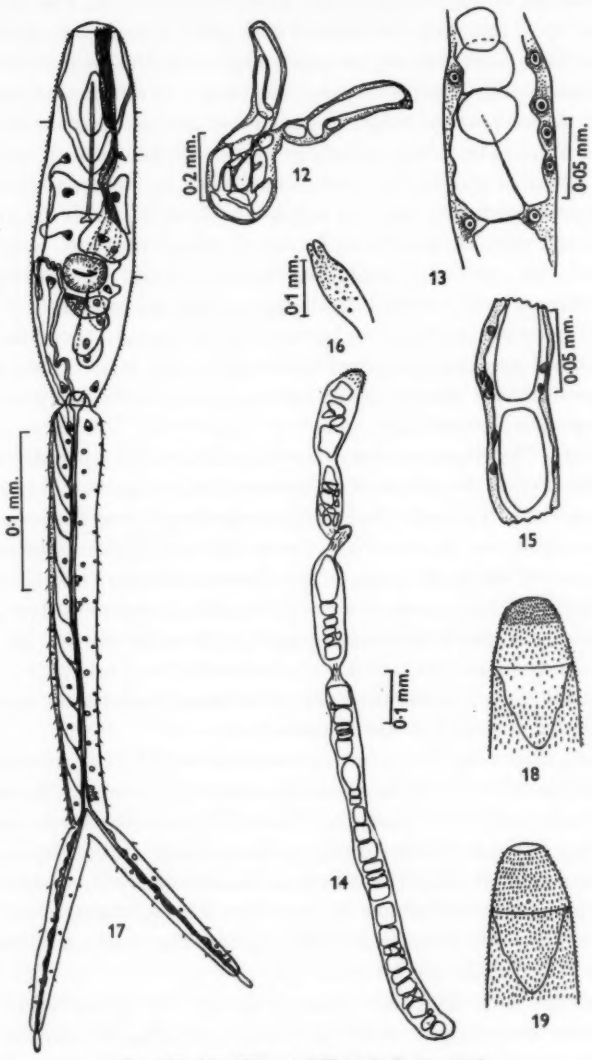
The spines. Spines cover almost the whole body, tail stem and the tail furcae. These spines are directed backwards and those on the body differ in distribution and size from those on the tail, being crowded on the dorsal side of the anterior part of the head organ. Following this area is a characteristic small area with scattered spines, which is followed by evenly distributed spines over the remainder of the body (Fig. 18). On the ventral surface the spines are evenly distributed (Fig. 19). Those on the tail stem are stouter, longer and more sparsely distributed than the body spines. The spines on the furcae are even less numerous than those on the tail stem. As far as I am aware, the small area with the scattered spines on the dorsal surface of the head organ is a character not previously described for schistosome cercariae.

The head organ. The head organ occupies almost the first third of the body. It is preceded on the ventral surface by an oral sucker. The posterior part of the head organ is strongly muscular, often contracts and appears as a separate ovoidal muscle surrounding the oesophagus and might be mistaken for a pharynx. It is possible that the structure which T. Oiso (1927) described as a pharynx in the cercaria of *Bilharziella yokogawai* is this muscle structure, since as La Rue (1938) stated: 'The pharynx according to Miller (1926) is a character of considerable value. Its absence characterizes the entire group of blood flukes, both adults and cercariae.'

The line of attachment of the posterior part of the head organ to the body wall is present and visible. The cystogenous gland or oral gland occupies the centre of the head organ and reacts like the posterior group of penetration glands to HCl-carmine. No opening could be seen from this structure to the outside, nor any extrusion noticed in the living cercariae. The head organ seems to disappear in the adult, in which no oral sucker is present.

Ventral sucker. The ventral sucker or acetabulum exists only in the carcaria and disappears in the adult. It is situated slightly posterior to the middle of the body and is more or less protrusible. Occasionally it could be seen extending outward in a side view of the cercaria. There are strong radiating muscles attached to the sucker. The sucker is very active in the measuring-worm-like movement of the cercaria.

Penetration glands. There are five pairs of unicellular penetration glands in the emerged cercaria (Fig. 17), set close together, starting from the area immediately anterior to the acetabulum and extending to the posterior part of the body. The first pair is located directly anterior to the acetabulum; the second pair, more or less elongated, surrounds the acetabulum and each gland is made up of three lobes. These two pairs together make up the first group. Similarity in staining reaction suggests the acidophilic nature, i.e. stain selectively with the alizarin vital stain (Gordon *et al.* 1934). Also, they usually take the eosin stain in permanent



Figs. 12–19. *Gigantobilharzia huronensis*

Fig. 12. Mother sporocyst, 15 days old.

Fig. 13. Body wall and some of the germinal materials in the lumen of a 14-day-old mother sporocyst.

Fig. 14. Daughter sporocyst, 18 days old, showing the spined anterior end.

Fig. 15. Body wall of 22-day-old daughter sporocyst.

Fig. 16. Birth pore of a 59-day-old daughter sporocyst.

Fig. 17. Cercaria constructed to show different structures. On the left showing excretory system and muscle cells in tail stem, on the right showing the penetration glands.

Fig. 18. Dorsal side of the anterior end of the cercaria showing the distribution of the spines.

Fig. 19. Ventral side of the same portion showing the distribution of the spines and mouth opening.

mounts. The nuclei in the two parts are generally spherical. The second group of glands is made up of the third, fourth and fifth pairs. They react similarly to stains suggesting the basophilic nature, i.e. stain blue with Delafield haematoxylin and take the carmine stain. Harris's haematoxylin (1:10 ammonium alum) was not satisfactory in this respect. It might be because of the presence of a high percentage of ammonium alum. The nuclei of this group are rather oval in shape compared with the first group of glands. The glands could not be differentiated into fine and coarse granulated groups as they all appear to be alike in living specimens. In specimens stained with Delafield's and eosin the three posterior pairs show coarse granules. Cercariae sectioned inside the infected snails show similar reactions, i.e. the suggestive oxyphily of the anterior group and the basophily of the posterior group. A sixth pair of penetration glands in the unemerged cercaria was not seen, not even in a good permanent stained specimen of this stage. Five pairs of ducts connect the penetration glands to the anterior part of the body with each duct ending in a separate, spined cup.

Digestive tract. The digestive tract consists of a mouth opening and a tubular oesophagus which ends about half-way between the eye spots and the acetabulum. The oesophagus ends in a bulb-like structure produced from the incomplete bifurcation of the oesophagus. In side view, the mouth opening could be seen in front of the muscular part of the head organ on the ventral surface. The pharynx is absent.

Excretory system. The excretory system, usually, consists of five pairs of flame cells, four pairs are in the body and one pair in the anterior part of the tail stem (Fig. 17). The body flame cells are distributed as follows: one pair lies anterior to the eyespots, the second is between the acetabulum and the eyespots, the third and the fourth are posterior to the acetabulum.

There are two collecting ducts, one on each side of the body extending from the posterior end to the level of the acetabulum, where they turn to the sides and then make another turn toward the posterior where they meet the anterior and posterior lateral collecting tubules. Before doing so the collecting ducts are provided with two ciliated patches each (Fig. 17). These can be differentiated from the flame cells by the flickering and the difference in structure. The collecting ducts unite in the posterior end of the body forming a small bladder. The structure generally known as the island of Cort could not be seen.

Anterior tubules each have two flame cells and the posterior tubules extend posteriorly to the anterior part of the tail stem. Attached to each of the posterior tubules are two flame cells in the body and one in the tail stem. The flame-cell pattern can be formulated as $2[(2)+(2+1)]$. The excretory system opens by a tubule protruding outward at the tip of each furca. Variations in the position of the flame cells and the looping of the tubules were noticed quite often. This might be caused by pressure from the cover slide and contractions of the body of the cercaria. A few cases of shifting of the position of the flame cells have been observed during this study. The second pair of flame cells were observed anterior to the eyespots beside the first pair, also the third and fourth pairs might come closer together in position. A few cases were observed of an extra pair of flame cells beside the third pair.

Nervous system. The nervous system consists of what is generally called the brain. This consists of two lobes connected by a commissure. The brain is situated ventral to the eyespots and stains deep blue with Delafield's haematoxylin.

The eyespots are found between the head organ and the acetabulum on the dorsal side. Each eyespot consists of a single lens directed toward the side, the pigmented granules, and the nerve cord, which is attached to the eye. The nerve cord can be seen with nile blue sulphate stain. A few hairs have been observed on each side of the body of the cercaria, attached to papilla-like structures (Fig. 17).

Primordium of the reproductive system. A mass of cells easily seen in stained specimens appears posterior to the acetabulum.

Behaviour. As soon as the cercaria emerges from the snail it moves actively for a short time and then becomes more or less quiet on the surface of the water. The cercaria attaches to the surface film with the body parallel to the surface and the tail hanging downward at various angles. The cercaria moves with tail forward, which is the usual method followed by similar cercariae. The forward motion is, apparently, effected by the rapid flapping of the furcae and the vibratile movement of the tail stem. The body of the cercaria seems to follow by a relatively more limited vibration. The cercaria also agrees with the other schistosome cercariae in the measuring-worm-like movement.

Emergence. The cercariae emerged from the snail host in the early morning around 5.30 a.m. (in a shaded and heated room in January). Most of the daily shedding takes place within about 4–5 hr., sometimes continuing until noon, in heavy infections.

Immediate emergence of the cercariae from the infected snails sometimes occurs as soon as the old water in the wide-mouth bottles is replaced by new water. This agrees with the observations of Soparkar (1921) on the cercaria of *Schistosomum spindalis*. This reaction appears also to be caused by a renewal of water in the present observations. R. E. Kuntz (1946), working on the effect of light and temperature on shedding of *Schistosoma mansoni* cercariae, has noticed, 'It would appear that any sudden alteration of temperature of the water in which the snails are immersed will induce a release of cercariae of *S. mansoni*.'

Cercariae were observed to emerge with the anterior part of the body coming first. This region contracts continuously back and forth until it frees most of the body. Then with a rapid vibrating movement it will free the tail. As soon as the whole body and the tail are liberated the cercaria turns over and moves with the tail forward. This method was used exclusively in all observed specimens. Faust & Meleney (1924) have stated for *Schistosomiasis japonicum* that, 'In all of our observations these cercariae have always emerged tail first.' Faust & Hoffman (1934) have stated for *Schistosoma mansoni* that 'Cercariae of *S. mansoni* emerge, tail first, from the infected snail, almost exclusively.' No case was observed in the cercariae of *Gigantobilharzia huronensis* that compared with the method observed by the above authors.

The cercaria as a dermatitis producing agent. Tests were made to determine whether the cercaria of *G. huronensis* is a dermatitis-producing agent. About twenty-five cercariae were placed in a 6 in. Petri dish, and the author's left arm

with the flexor surface down was immersed in the water containing cercariae. After 5 min. the arm was removed to dry, and at the same time six cercariae were pipetted separately on six different dry spots of the same arm. After about 20 min. strong itching was felt on several places of this arm. Ten minutes later five red spots became apparent. In addition, four reddish spots appeared shortly afterwards in the area of the six pipetted drops. The spots enlarged in size and 2 days later they became nodular in shape. After 2 weeks they were still detectable. A repetition of this experiment some 9 weeks later but using the right arm produced a similar reaction. No pus was produced in either case. These tests showed that cercariae of *G. huronensis* are a dermatitis-producing agent, and so a new member had been added to the dermatitis cercariae group in the United States.

DISCUSSION

The species *G. huronensis* is placed under the subfamily Bilharziellinae Price, 1929, and the genus *Gigantobilharzia* Odhner, 1910, because of the similarity in form between the male and female, i.e. both having slender bodies. It has also short, paired intestinal caeca, long common caecum, numerous testes situated along the course of the common caecum, gynaecophoric canal reduced to a short groove situated in the anterior part of the body, no suckers, and the uterus may contain one or several eggs.

Odhner (1912) gave a diagnosis of the genus *Gigantobilharzia*, which he based on his description of a single species, *G. acotylea* Odhner, 1910. Price (1929) gave the generic diagnosis and stated erroneously 'cirrus pouch absent', which should read, 'cirrus pouch present', because the same author in the species description stated: 'terminal portion of genital system (Endapparat) consists of a cirrus pouch containing...'. This statement agrees with the original description.

Since five more species of the genus *Gigantobilharzia* have been described, the existing generic diagnosis is no longer valid and a revised diagnosis should replace it. It is difficult to define precisely the genus *Gigantobilharzia* because of inadequate descriptions of some species. This apparently is due to failure of some authors to secure sufficient specimens for detailed studies.

In almost all of the known species of the genus *Gigantobilharzia* the body was described as long, filamentous or thread-like. Male and female are, more or less, similar in form with the exception of *G. actoylea* Odhner, 1910, in which the male is somewhat flattened. None of the species have spines or any kind of cuticular armaments. However, small papillae were observed on the anterior end of both sexes in *G. huronensis*. Also, in the same species, small wart-like protuberances were observed scattered irregularly along the body of both sexes. These protuberances might be caused by the fixative. The acetabulum is absent from both sexes in all the described species of the genus. The oral sucker is present in one sex (apparently only the male of *G. monocotylea* Szidat, 1930) or in both sexes (in *G. gyrauli* Brackett, 1942), or the oral sucker is absent (*G. acotylea* Odhner, 1910; *G. egreta* Lal, 1937; *G. lawayi* Brackett, 1942; and *G. huronensis* Najim, 1950). Brackett (1942) erroneously indicated the presence of an oral sucker in the type species, *G. acotylea* Odhner, 1910.

The digestive system is generally similar in all the species where this system was described. Paired intestinal caeca in the male are anterior to the gynaecophoric canal, or anterior to this level, when the gynaecophoric canal is absent. The intestinal caeca in the female unite directly posterior to the seminal receptacle. Mouth is terminal or subterminal. The seminal vesicle and the adjacent organs (Endapparat) show wide differences—from a simple sac to a more or less complicated structure as was previously mentioned.

The gynaecophoric canal is absent or imperfectly formed, a situation which is not unfamiliar among the members of the subfamily Bilharziellinae. McMullen & Beaver (1945), in discussing this structure in the subfamily Bilharziellinae, referred erroneously to *Pseudobilharziella filiformis* Szidat (1939), while, it appears, they wanted to refer to *G. monocotylea* Szidat (1930), because the gynaecophoric canal is present, without doubt, in *P. filiformis* Szidat (1939), and the statement and argument of the two authors rather apply to *G. monocotylea* Szidat, 1930. Whether there is a gynaecophoric canal in *G. monocotylea* which Szidat might have overlooked, as McMullen & Beaver (1945) pointed out, or whether it is just an enlargement in the body as the figure showed, cannot be determined unless the specimens themselves are re-examined. Szidat's statement of the lack of a gynaecophoric canal in his species *G. monocotylea* will have to be accepted.

The male genital pore opens at the beginning of the gynaecophoric canal, or at this level when the gynaecophoric canal is absent. In some species the opening is described on the left of the midline.

The testes of the species of *Gigantobilharzia* differ in shape, size and arrangement. The testes in *G. acotylea* are small, oval-shape, start shortly posterior to the gynaecophoric canal and are set on both sides of the vas deferens. Szidat (1930) did not mention much about this structure in *G. monocotylea*, nor do his diagrams show clearly these structures. In *G. egreta* Lal, 1937, the testes are rather large, round to oval in shape, and lie on both sides of the common caecum. The testes of *G. gyrauli* Brackett (1942) show no regular shape and start shortly posterior to the gynaecophoric canal. In *G. lawayi* Brackett (1942) the testes are wider than long, arranged in tandem, and start farther forward than the posterior end of the gynaecophoric canal. The shape of the testes in *G. huronensis* is, more or less, like those in *G. egreta* Lal, 1937, but the latter are much larger in size. The testes of *G. huronensis* start from a point farther forward than the posterior end of the gynaecophoric canal. The testes in all the described species occupy the greatest portion of the body length and extended posteriorly to a point close to the end of the body. From the above discussion it may be concluded that the testes are different in shape, some are round to broadly oval, others are oval and some are irregular in shape. The testes start from a point shortly posterior to the posterior end of the gynaecophoric canal (when this structure is present) or farther forward than the posterior end of the gynaecophoric canal. In all species the testes are numerous.

The ovary of the described species of *Gigantobilharzia* is in accord with what has been described for the subfamily Bilharziellinae, being an elongated, more or less spiral, organ. The connexion of the ovary to the oviduct and the latter to the seminal

receptacle has been discussed previously. However, similar connexions to that of *G. huronensis* are found in *Ornithobilharzia emberizae* Yamaguti, 1941, but in the latter species Laurer's canal is located posterior to the seminal receptacle, while in *G. huronensis* the canal is found between the seminal receptacle and ovary. The female genital pore opening is close to the mouth opening, shortly posterior to the latter opening in almost all the described species. McMullen & Beaver (1945) suggested re-examination of the location of the female genital opening in *Gigantobilharzia*. This work confirms the previous observations on other species of the genus, and it can be stated that the female genital opening is located in a characteristic position in almost all the members of the genus *Gigantobilharzia*.

The posterior end of the body is blunt, with the exception of *G. acotylea* Odhner, 1910, which has elaborated lateral expansions. However, some species show a sort of flattening at this end. Tanabe (1951) designated *Cercaria sturniae* as *Gigantobilharzia sturniae* Tanabe, 1948 n.comb. Since Tanabe's description of the new species is scanty and inadequate, not much can be done with this species. However, concerning the presence of an acetabulum Tanabe stated that there was 'an anteriorly placed genital pore and acetabulum, the latter being very indistinct or possibly absent'. Since the absence of the acetabulum is one of the determining characters in the genus *Gigantobilharzia*, in which it has not been reported in any of the other described species, Tanabe's species should be re-examined. From the above discussion the following generic diagnosis is submitted:

GENERIC DIAGNOSIS. *Gigantobilharzia* Odhner, 1912 (emended). Bilharziellinae: male and female elongated, filiform; or the male may be somewhat flattened. Cuticle without spines, acetabulum absent, oral sucker present or absent.

Male: Gynaecophoric canal, if present, short, situated in anterior part of body; digestive system similar to that of *Bilharziella*; testes originate posterior to or anterior to the posterior end of the gynaecophoric canal. Cirrus and cirrus pouch present or absent. Genital pore situated at anterior end of gynaecophoric canal or at about this level when the gynaecophoric canal is absent.

Female. Ovary moderately long and spiral; vitelline follicles occupy most of the body length. Uterus contains a single or several eggs; genital pore shortly posterior to the mouth opening; eggs oval to almost spherical. Adults found in birds.

Type species. *Gigantobilharzia acotylea* Odhner, 1910.

Key to the species

1.	Gynaecophoric canal present	2
	Gynaecophoric canal absent	5
2.	Oral sucker present	**G. gyrauli** Brackett, 1942
	Oral sucker absent	3
3.	Male somewhat flattened 140–160 mm. long by 0·25–0·35 mm. wide, female filamentous 30–35 mm. long by 0·1–0·20 mm. in diameter	**G. acotylea** Odhner, 1910
	Male and female filamentous, female longer than male	4
4.	Oesophagus in male 0·22–0·30 mm. long; female about 10 mm. long.	**G. lawayi** Brackett, 1942
	Oesophagus in male; female 18–29 mm.	**G. huronensis** Najim, 1950
5.	Male and female described, oral sucker present in male	**G. monocotylea** Szidat, 1930
	Male only described, oral sucker absent	**G. egreta** Lal, 1937

The miracidium of *G. huronensis* is peculiar in having an unciliated area around the middle of the body, a character which, as far as can be ascertained, is not found in any other schistosome miracidia. Since the miracidium of this species agrees closely with other known schistosome miracidia in the number and arrangement of epidermal plates, the gap round the middle of its body is apparently the result of shortening in the length of the plates, especially those of the second row. The plates of this miracidium were easily counted in well-flattened preparations treated with silver nitrate. A small amount of the mounting material was used in order to allow the cover-glass to flatten the specimens, otherwise it was difficult to determine the right number of plates.

Looss (1892), working on the miracidium of *Amphistomum subclavatum* (Rud.), found that the gut differentiates in early stages of embryonic development. It starts as a few cells in the fore-body of the miracidium and later forms a flat layer of cells surrounding a space inside. The cells break up and their cytoplasmic material fills the entire lumen. This situation also probably occurs in the miracidium of *Gigantobilharzia huronensis*, and the nuclei which are present in the lumen of the gut, like those of *Amphistomum subclavatum*, are the remains of the broken cells.

The location of the bristle patches in this miracidium is similar to that of the miracidium of *Schistosomatium douthitti* studied by Price (1931). Nevertheless, the location of these structures in the miracidia of *Gigantobilharzia huronensis* is different from the arrangement of the bristle patches in some human schistosome miracidia. The observations of Looss (1866–1901) and Brock (1893) on the miracidium of *Schistosomum haematobium* agree on the presence of interruption in the body cilia. According to Looss this interruption is caused by two circles of fine papillae. The papillae are arranged around the body shortly behind the head and slightly posterior to the middle of the body. The observations of Brock indicate the presence of the bristles, apparently, in the region behind the middle of the body. My observations on the miracidium of *Schistosoma mansoni*, both living and in silver nitrate preparations, confirm the observations of Brock (1893). It was found that the majority of the bristle patches are posterior between the second and third row of epidermal plates. However, few circles were seen, in silver nitrate preparations, between the first and second row. These few circles, which look similar to the bristle-patch circles, were not determined in the living specimens because of the death of the only mouse infected. It is believed that these few circles in *S. mansoni* probably represent a few bristle patches in this area.

The cercaria of *Gigantobilharzia huronensis* falls in Sewell's (1922) 'spindalis' group and H. M. Miller (1926) group B of the furcocercous, apharyngeal, brevifurcate distome cercariae. Sewell listed two cercariae under the 'spindalis' group, namely, *Cercaria spindalis* and '*C. Indicae* XLVII'. H. M. Miller (1926) added *Cercaria* B (Kemp) to Sewell's *spindalis* group on the basis of similarity of the number and the character of the penetration glands and changed the name *spindalis* to group B in his classification.

The cercaria of *Gigantobilharzia huronensis* can be differentiated from the cercaria of *Schistosomum spindalis* in being larger in size; no differentiation of the penetration glands in the living specimens of the cercaria of *Gigantobilharzia*

huronensis; absence of the eyespots in *G. spindalis*. In behaviour, *Cercaria spindalis* hangs in water with the head down and the tail turned upwards, while *C. huronensis* hangs with body parallel to the surface.

C. huronensis differs from cercariae '*Indicae* XLVII' in being slightly smaller than the latter and also in the proportional size of the parts, i.e. the tail in *C. indicae* being shorter than the body; in *C. indicae* the penetration glands are differentiated into coarse and fine granulated groups; the first anterior pair of flame cells starts from a point posterior to the eyespots; the body being armed with spines in the anterior two-thirds, but the posterior third appears to be devoid of armature and finally differs in the mollusc intermediate host, which is an aperculate, *Melanoides tuberculatus* Muller, for '*Cercariae Indicae* XLVII' while *Gigantobilharzia huronensis* has a pulmonate intermediate host.

E. L. Miller (1935) reported *Cercariae pteractinota* from *Physa gyrina* and *Helisoma trivolvis*. The author did not give the number of the penetration glands, neither did he give the pattern nor the number of the flame cells of the cercaria. However, Miller's cercaria differs from the cercaria of this species in that its tail is three times as long as its body. *Cercariae pteractinota* also possesses prominent pigmented masses one on either side of the oral sucker near its posterior end, it hangs in the water with its head downward, and finally, it possesses two germ masses instead of one.

Porter (1938) described *C. elandsiae* from South Africa and determined it as a longifurcous cercaria. But Porter's *elandsiae* is rather a brevifurcate since its furcae are slightly less than half the length of the tail stem. *C. elandsiae* possesses 2 (4 + 1) flame cells, and its size is generally close to the size of the cercaria of this species. However, *C. elandsiae* has only four pairs of penetration glands, the arrangement of the flame cells are different, it does not hang to the surface film of the water, and, finally, the intermediate host is *Lymnaea natalensis*.

The cercaria of *Gigantobilharzia huronensis*, in fact, could be easily confused with the cercaria of *Schistosomatium douthitti* (Cort) (Price, 1931), and the cercaria of *Gigantobilharzia gyrauli* (Brackett, 1940). The three cercariae are similar in behaviour and size. The cercaria of *G. huronensis* can be differentiated from the cercaria of *Schistosomatium douthitti* by the number of flame cells. The penetration glands, in *S. douthitti*, are differentiated into anterior coarse granular group and a posterior fine granular group. Also in *S. douthitti* the absence of the fin folds from the furcae and the emerging time in the evening differ from *Gigantobilharzia huronensis* which emerges in the morning and has five folds.

In comparison, the cercaria of *G. gyrauli* has more flame cells, with a pattern of 2 (3) + (3 + 1). The penetration glands are of two kinds, fine and coarsely granular, but they are reversed from the arrangement of *Schistosomatium douthitti* as indicated by Brackett (1940). The digestive system is more developed in *gyrauli*, the intestinal caeca extend posteriorly to the level of the acetabulum.

It is not difficult to differentiate the cercaria of *Gigantobilharzia huronensis* from the cercaria of *B. polonica* (Kow) (Szidat 1929). The latter cercaria is larger in size, has six pairs of penetration glands consisting of two groups, coarse and finely granulated, and finally the number of flame cells is '2 (6 + 1)' in *Cercariae polonica*.

C. littorinalinae Penner (1950) shows similar behaviour to that of the cercaria of *Gigantobilharzia huronensis*, but it can be differentiated by size, the former is larger and also has more flame cells. The digestive system is more developed, and finally, the cercaria develops in a marine snail *Littorina planaxis philippi*.

SUMMARY

1. The life cycle of *Gigantobilharzia huronensis* Najim, 1950, was determined experimentally. The snail, *Physa gyrina* (Say), served as the intermediate host. The natural definitive hosts were goldfinches (*Spinus tristis tristis*) and the cardinals (*Richmondena cardinalis*). Chicks and canaries served as susceptible hosts in the laboratory

2. The shortest time recorded for the hatching of eggs in water is about 20 min. Cercariae were liberated from the snail as early as 24 days after exposure. Eggs were found in the faeces of the definitive host 31 days after the first exposure to the cercariae.

3. Adult worms are elongate and filiform. Females are longer than males, and in both sexes the oral and ventral suckers are absent. The cuticle has no spines. A short gynaecophoric canal is present. Up to 300 testes are present in the male and the cirrus is spiny. Laurer's canal is present and vitelline follicles fill most of the body of the female. Eggs are spherical to very broadly ovoid in shape and averaged 93μ in length by 88μ in width. The uterus contains from one to several eggs at a time, and the genital pore is located shortly posterior to the mouth opening.

4. The miracidium has a peculiar wide gap between the second and third rows of epidermal plates. The body covering consists of twenty-two epidermal plates arranged in four rows of 6:9:4:3. The mother sporocyst has an elongate, sac-like body. A birth pore is present in the mature mother sporocysts. The daughter sporocyst also has an elongate body, but the anterior end is covered with spines. A birth pore is present in the mature daughter sporocysts.

5. The cercaria is an apharyngeal, furcocercous, brevifurcate, distome with pigmented eyespots; the body averaging 240μ in length by 72·5μ in width; the tail stem averaging 268·5μ in length by 30μ in width; the furca averaging 146·8μ in length by 15μ in width. The furcae have fin folds. The body and tail are covered with spines. There are five pairs of penetration glands and an oral gland. The excretory system is usually 2[(2)+(2+1)]. The cercaria attaches to the surface film of the water with the body parallel to it and the tail hanging downward at different angles. The cercaria is a dermatitis producer in human skin.

Grateful acknowledgement is made to Dr Arthur E. Woodhead under whose supervision and guidance this study was undertaken. Sincere thanks are expressed to Dr George R. La Rue for valued and helpful suggestions; to Dr Robert B. Short for providing the first materials with which the problem was started. Thanks are due to Dr Henry van der Schalie for identifying the snail host; to Dr Harry W. Hann for identifying some of the bird hosts. This study was supported by the Ministry of Education, Iraq.

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(*MS. received for publication* 5. VIII. 55.—Ed.)

LIFE CYCLE OF *AUSTROBILHARZIA TERRIGALENSIS* JOHNSTON, 1917

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(With 9 Figures in the Text)

The present paper reports the life cycle of the schistosome, *Austrobilharzia terrigalensis*, the cercaria of which has been shown to cause experimental dermatitis in man (Bearup, 1955). The naturally occurring dermatitis from the salt water of Narrabeen Lagoon, New South Wales, is probably due to the same cause.

The adult worm was described by Johnston from the seagull, *Larus novaehollandiae*; the type locality was Terrigal, near Sydney. A description of the cercaria appeared in a previous paper (Bearup, 1955) and the cercaria was tentatively named *Cercaria variglandis* Miller & Northup, subspecies *pyrazi*, new subspecies; the adults had not then been found. Now that the adult worms have been recovered after experimental infections of gulls and other birds and have been identified with *Austrobilharzia terrigalensis*, the subspecies *pyrazi* lapses.

No difference was found, by the present author, between worms from experimental hosts and those occurring in natural infections in seagulls. The differences between these worms and those described by Johnston are in the female and are thought to be due to his defective material.

MATERIAL AND METHODS

The cercariae used for experimental infections of birds were obtained from *Pyrazus australis* Quoy & Gaimard,* which is probably the commonest mollusc in Narrabeen Lagoon. The snails were collected from the sandflats at low tide and were in greatest numbers in and around beds of the ribbon weed, *Zostera*. From 4 to 6% of them carry this forktail cercaria; the higher rates occur in the hot months at the end of the year.

The snails are kept in a small quantity of lagoon water in flat-bottomed glass tubes. Trays of them are placed in the light of a window facing the sun for 2 or 3 hr. and then examined for cercariae. Naturally emerging forktail cercariae make to the top of the water and lie flat and almost motionless in the surface layer. They stick tight to any object with which they come in contact, using their powerful ventral suckers for this purpose. For this reason Pasteur pipettes are almost useless to transfer them as they stick fast to the surface of the glass.

Birds for experimental infection are stood in a few millimetres of water with cercariae from several snails for about an hour. Cream cans are convenient vessels as the birds can be left in them without any need for restraint.

* According to Iredale (1936) the genus is *Velacumantus*.

Young seagulls were collected as nestlings from the breeding grounds on small islands off the coast, where *Pyrazus australis* does not occur. Birds other than seagulls were obtained from commercial breeders in the city.

Post-mortem, the easiest way to look for mature worms or for their ova, is by compressing the organs between two glass plates. A large compressorium such as is used for searching for *Trichinella spiralis* larvae is excellent for this purpose as it may be examined under the low magnification of a dissecting microscope. Eggs can be seen easily as the dead ones are calcified and the living ones surrounded by a clear halo several times their own diameter.

The worms are examined alive in the relaxing solution recommended by Balenger & Neuzil (1953), consisting of menthol, 0·25 g.; Tween 80, 5·0 g.; water to 100 c.c. The worms soon become motionless, and under slight compression by a cover-slip nearly all details of the anatomy can be seen much better than in preserved and stained specimens.

DESCRIPTION OF ADULT WORMS

Males and females of experimental infections are found in the veins of the portal system, usually in coupled pairs, with the female enclosed in the gynaecophoric canal of the male. The worms are distributed in the larger veins of the intestine between the levels of the pancreas and the caeca 2–3 cm. from the rectum. Single worms of either sex are found occasionally with males predominating.

Eggs with miracidia were found in the faeces of two birds, a pigeon and a budgerigar, 18 days after exposing them to cercariae. At this stage most of the worms are still in the liver; a pigeon killed at 32 days after infection had seven worms in the intestinal veins and seventy-nine still in the liver. About half of the latter were coupled and were probably ready to migrate. The rate of loss of worms seems to be high. One mature bird had eggs in the faeces at the time of capture; 7 months later neither worms nor ova were found in liver or gut. Another gull had schistosome ova 4 months after experimental infection, but 7 weeks later neither living nor degenerating worms could be found, although viable ova were still present in the liver and intestinal wall.

Seagulls (*Larus novae-hollandiae*) from the Sydney district are nearly all (12 of 14), naturally infected with *Austrobilharzia terrigalensis*, and no other schistosome was found in them during this study. As a rule infections with mature worms are light, in natural or in experimental hosts. Experimental infections have been established in three of three budgerigars (*Melopsittacus undulatus*), in three of five pigeons (*Columba livia*) and in four of five seagulls (*Larus novae-hollandiae*), but not in three domestic ducks or in three laboratory mice.

The excretory system in both sexes consists of a thick-walled excretory vesicle connected to the posterior end by a short duct and to two main lateral ducts which extend to the anterior end. The complex system of subsidiary branches from these ducts could not be followed and the only flame cells seen were one on each side of the oral sucker. The excretory vesicle is cylindrical to triangular; the latter shape is more usual and the cylinder may be brought about by lifting the anterior wall of

the triangle by muscular action. At the posterior ends of the longitudinal vessels are two patches of cilia.

Females are longer and thinner than males and have less prominent oral and ventral suckers. The male has a well-developed gynaecophoric canal which extends from the posterior end of the body to the level of the ventral sucker. Both sexes have longitudinal striae on the cuticle; they are more prominent in the female but can be easily seen on the infolded edges of the ventral side of the male.

Specimens of worms from experimental and natural infections have been deposited with the Australian Museum, Sydney: W 3593, stained slide of male and female from experimental infection in *Columba livia*; W 3594, stained slide of male and female from *Larus novae-hollandiae*; W 3595, specimens in spirit from experimental infection in *Columba livia*.

Males (Fig. 1)

The mean lengths of mature living males are almost identical in natural and experimental infections; they are respectively 3·4 mm. (seven worms measured) and 3·5 mm. (ten worms). Maximum width in the middle region is 0·4 mm. The body shows a definite antero-posterior curve towards the ventral side. Both suckers are about the same size, 0·2 mm., and have fine spines inside. The ventral sucker is stalked and is about one-sixth of the body length from the anterior end.

The gynaecophoric canal consists of the ventrally folded borders and is deepest about the middle of its length. Usually it appears to end at the posterior border of the ventral sucker, but it is possible that it extends around the stalk of the sucker and that this extension is hidden from a ventral view. On one occasion, from a lateral view and under pressure from a cover-slip, the ventral sucker was displaced posteriorly and the canal then seemed to extend around the base of the sucker (Fig. 9). No other male worm was seen, in either natural or experimental infections, in which the canal extended anterior to the ventral sucker. The canal is lined with numerous transverse ridges each bearing several rows of prominent spines. The nerve ring is 0·27 mm. from the anterior end.

The mouth opens at the base of the oral sucker and is followed by a thick-walled oesophagus which widens posteriorly where it is surrounded by a group of gland cells. At the anterior level of the ventral sucker the intestine divides into two branches which diverge to run dorso-ventrally and do not reunite until they reach the third quarter of the body length. Here there are one or more anastomoses; the exact number is usually uncertain because of the many overlapping diverticula from both caeca. The testes vary in number from twelve to twenty; they are rounded to oval, with a diameter from 0·02 to 0·03 mm. They extend in an irregular double row in the second quarter of the body, midway between the intestinal caeca. A single vas deferens runs to a genital opening within the gynaecophoric canal just (0·10 mm.) behind the ventral sucker.

Females (Figs. 2 and 3)

The mean lengths of mature living females in natural infections of seagulls are 5·0 mm. (Seven worms measured) and in experimental infections 4·6 mm. (ten worms). The greatest width is 0·25 mm. in the middle, decreasing to one-half of this anterior to the ovary.

Oral sucker, 0·05 mm., lined with fine spines. The muscles of the oral sucker are poorly developed and the sucker sometimes cannot be seen from a lateral view. In such cases the cup of the sucker can be seen when the worm is rolled to give a ventral view. Ventral sucker 0·065 mm., stalked, with fine spines inside, about one-sixteenth of body length from oral sucker. Longitudinal striae extend from behind the oral sucker to the yolk glands where the dark background makes them invisible. Mouth opening in the base of the oral sucker. The oesophagus widens posteriorly, and this part is surrounded by gland cells; at the anterior border of the ventral sucker it divides into two caeca. These reunite behind the ovary and continue a sinuous course to near the posterior end.

Ovary spiral, about 0·8 mm. long in the coiled position. Usually it lies across the equator and mostly in the anterior half, but the state of extension of the mobile pre-ovarian part of the worm alters this relationship. The oviduct runs forward from the posterior end of the ovary and joins the yolk duct. Vitellaria fill the posterior half of the worm; the single duct joins the oviduct about 0·15 mm. from the ventral sucker. Then follows the uterus containing no more than a single ovum, a short vagina and the genital opening just posterior to the ventral sucker.

Ova

Ova in the uterus are smaller than those in a later stage of development in the liver or gut. Often they are round, sometimes drawn out to a blunt point at one or both poles. Many ova in the liver and gut wall are dead and partly or completely calcified. Living ova in the tissues are surrounded by a clear zone about twice their diameter; this is probably a zone of digestion caused by secretions from the egg. All stages of development up to the fully formed and motile miracidium are found in the liver and intestinal wall.

The eggs (Fig. 4) in the liver, intestinal wall and faeces are oval and usually have a terminal spine bent as a J; sometimes this is straight or absent. Mean measurements of twenty fresh ova in the gut wall was $88 \times 83\mu$; the spine adds $7–10\mu$. The ovum in the uterus is about 55μ and has no spine. The fully developed miracidium is bent into U-shape within the egg shell. When about to hatch the eggs swell to as much as 130μ and the spine then disappears.

Miracidia

When eggs are placed in lake water the miracidia (Fig. 5) become very active and hatch within a few minutes. In one observation, a split occurred close to the spine and the larva escaped in a few seconds. The larvae change shape continually, but measure about $150 \times 50\mu$. They swim forward strongly, except when investigating patches of mucus or the like by probing with the anterior end.

The epidermis is uniformly covered with cilia about 10μ long, except for the anterior tip and two gaps on the lateral borders which probably represent the interstices of the epidermal cells. The anterior third of the body is occupied by two gland cells and by the primitive gut.

The excretory system consists of two pairs of flame cells, one pair anterior and one pair posterior in the body; both are connected by capillaries to a common duct which opens to the postero-lateral border. There are some scattered groups of germ cells in the posterior half of the body.

Sporocysts (Fig. 6)

Attempts to infect young *Pyrazus australis* with miracidia have not been successful, and no mother sporocysts have been found by dissecting the mantles or digestive glands of infected snails.

The daughter sporocysts are variable in size, up to 0·7 mm. long. They are elongate-oval, sometimes with irregular constrictions which are probably due to pressure from other larvae developing alongside them. Large numbers of sporocysts are the rule, even in snails which had been in captivity for 6 weeks and which had emitted cercariae for that time. A channel leading from the exterior towards the body cavity may represent a birth pore although no cercariae were seen to escape from it. In some smaller sporocysts a plug of brownish material covers the internal face of this structure and motile cercariae push against it as if trying to escape in that direction. Some sporocysts are filled with mature and motile cercariae with very few 'germ balls' and these might contain twenty fully grown cercariae. Smaller sporocysts have a greater proportion of immature cercariae. The sporocysts project slightly above the surface of the gland and in a heavy infection give it a furred appearance. In such infections the whole of the gland appears to be replaced by sporocysts and they are found in other organs of the reproductive to digestive systems as well.

Cercariae

The cercariae (Fig. 7) are binoculate, apharyngeal, furcocercous, with minute spines on the body, tail stem and furcae. Measurements in microns of twenty-five larvae (recently killed in hot formalin) are as follows (mean, standard error of mean, and range are given in that order): length of body 235 $\pm$ 3·0 (203–270); breadth of body, 69 $\pm$ 1·0 (61·5–80); length of tail stem, 265 $\pm$ 4·9 (228–330); length of furcae, 148 $\pm$ 3·4 (116–170). No fins could be seen on the furcae, tail stem or body. A thin epithelium covers the body and furcae; around the tail stem it is thicker.

The anterior organ is well defined by a muscular wall which thins out anteriorly and merges with the epithelium. The extreme tip of the anterior organ when fully extended shows several pointed papillae and the ends of the ducts of the penetration glands; when the tip is retracted a V-shaped depression forms on the ventral side enclosing the papillae and the orifices of the ducts. The mouth opening is ventral, about 40μ from the anterior end, and passes into a simple oesophagus and a gut which ends just behind the eyes with two short caeca. There

is no pharynx. The gland cells are disposed in relation to the acetabulum as follows: one pair anterior, one pair lateral and four pairs posterior. The ducts arise laterally and continue forwards and inwards to the level of the eye-spots, where their lumen is considerably reduced. They then turn sharply outwards and dilate until another constriction occurs at about the level of the posterior border of the anterior organ. As they pass through the muscular wall there is a third constriction; from here they run a sinuous course to the anterior border. In haematoxylin-eosin sections the glands and their ducts are strongly eosinophilic. The two anterior pairs of glands have finer granules than the four posterior pairs.

The ventral sucker is very protrusible; the mid-point is about 0·13 mm. from the anterior end; the mean diameter is 0·028 mm. Many muscle fibres radiate from it towards the dorsal side of the body.

The nerve ring is a broad band of tissue between and extending laterally just beyond the eyespots. The eye-spots are cup-shaped and consist of about sixty small, discrete, brownish black granules. They are on the dorsal surface about 0·09 mm. from the anterior end.

The primitive gonads are a triangular group of cells posterior to the ventral sucker and near the ventral surface.

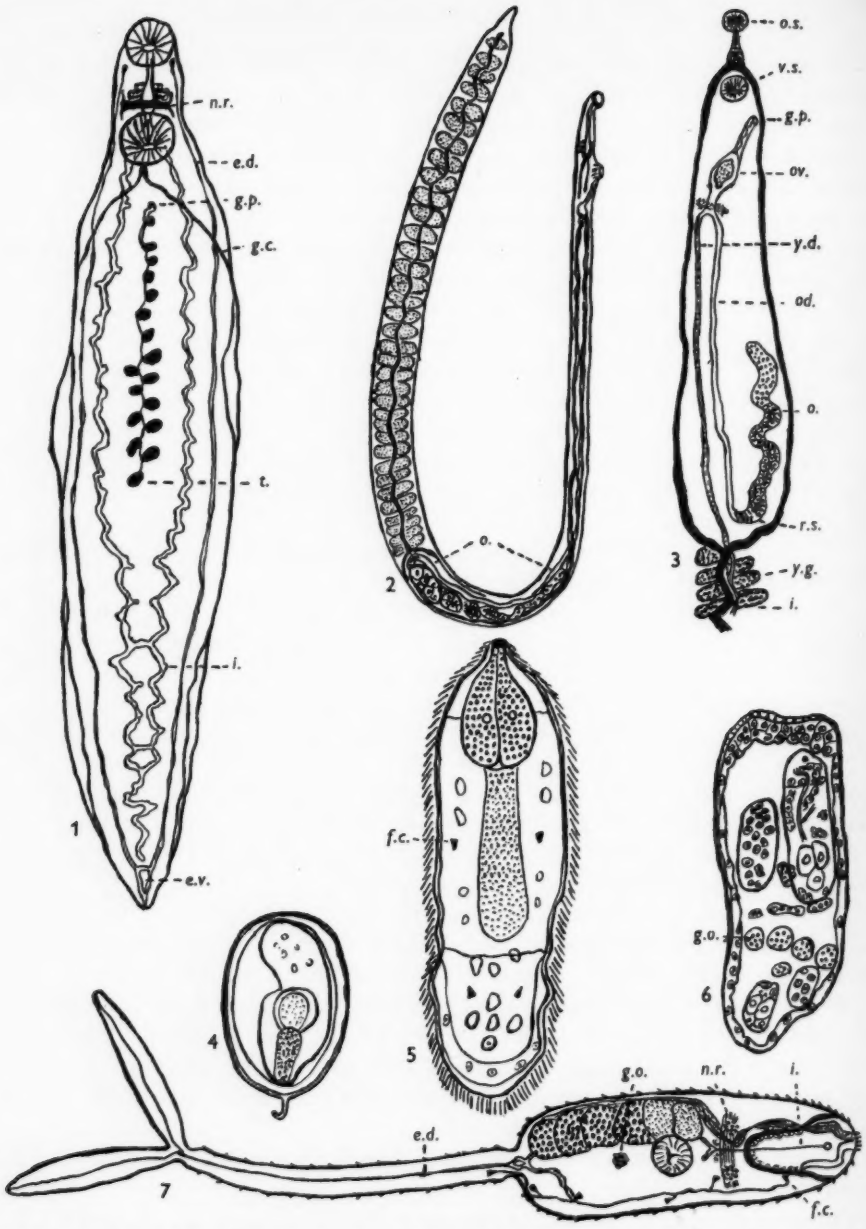
The excretory system has no definite bladder. Two main ducts pass antero-laterally and, before reaching the acetabulum, they divide into anterior and posterior collecting tubes. Three flame cells are connected to each tube. The six pairs of flame cells are distributed as follows: postero-lateral to the anterior organ, eyespots and ventral sucker, half-way between the ventral sucker and the posterior end, near the posterior end and at the root of the tail. Only one ciliated patch could be found, it was difficult to see as it lies below the fourth flame cell from the anterior end. In the tail the two main excretory ducts continue posteriorly around the island of Cort and then fuse. At the base of the tail the single duct redivides and single ducts continue to the tips of the furcae.

A small proportion of naturally emerged cercariae have one of the pair of most anterior flame cells and its duct subdivided into two so that the flame cell formula is irregular, with six on one side and seven on the other.

About 4 % (111 in 2600) of *P. australis* collected from Narrabeen Lagoon during the past 12 months have been infected with this cercaria. Infections have been present at about this rate throughout the year with figures rising to 6 % in the hot months of December and January. About half the infections are discovered by spontaneous emergence of the cercariae.

P. australis is common on sand flats in estuaries and coastal lagoons along the coast of New South Wales.

Cercarial emergence can be stimulated by bright sunlight and warmth. In summer at room temperature the cercariae emerge in greater numbers around midday. The cercariae are active swimmers and most of them quickly make for the water surface, where they lie with the tail bent laterally, so that the furcae approach the body. At the same time the tail is shortened and numerous transverse wrinkles give it a shrivelled appearance. In swimming, the tail precedes the body; movement is effected by a rapid lateral vibration of the tail stem, while the



Figs. 1–7. *Austrobilharzia terrigalensis*. 1. Male. Ventral view, under slight compression by the cover-slip. 2. Female. Lateral view. Part of intestine omitted. 3. Female. Diagram of reproductive system and part of digestive system. 4. Ovum. From faeces. 5. Miracidium. 6. Sporocyst. Section of digestive gland of *Pyrazus australis*. 7. Cercaria. Gland cells and excretory system omitted on opposite sides. (Main features of 2, 4 and 6 drawn by camera lucida; 1, 3, 5 and 7 are composite drawings.) *e.d.* excretory duct; *e.v.* excretory vesicle; *f.c.* flame cell; *g.c.* gynaecophoric canal; *g.o.* germinal cells; *g.p.* genital pore; *i.* intestine; *n.r.* nerve ring; *od.* oviduct; *o.s.* oral sucker; *o.* ovary; *ov.* ovum; *r.s.* receptaculum seminis; *t.* testis; *v.s.* ventral sucker; *y.d.* yolk duct; *y.g.* yolk glands.

furcae are held rigid and at right angles to the axis of the body and tail stem. The tail is easily detached from the body and both are capable of independent movement, the body by creeping movements using the head organ and acetabulum and the tail by swimming.

DISCUSSION

Austrobilharzia terrigalensis as described by Johnston (1917) differs in several features from the description given in this paper. The important differences in Johnston's material are: (1) the absence of the oral sucker in the female and (2) the posterior position of the ovary (postequatorial) and the yolk glands (occupying posterior third of the body).

The differences given under (2) are probably both related to the state of extension of the more mobile part of the worm anterior to the yolk glands; in living worms it can be seen to extend and contract quite enough to account for the different relative measurements of Johnston's material and my own. In the relaxed state induced by menthol the ovary usually extends across the equator, but the greater part of it is on the anterior side; as the yolk glands commence just behind the ovary, this means that the yolk glands occupy nearly half the body.

The presence of the oral sucker in the female can best be seen while the worm is still alive. This sucker is poorly provided with muscle fibres, but it is always set off from the rest of the body by its shape (Fig. 8). Further evidence of its nature is that the inner surface is provided with fine spines (very small but visible in living material) resembling those of the ventral sucker, and that the oesophagus opens into the base of it. Several hours after the worm has been collected, or when it has been fully relaxed by menthol, the oral sucker is so poorly defined that it is hard to distinguish as a sucker.

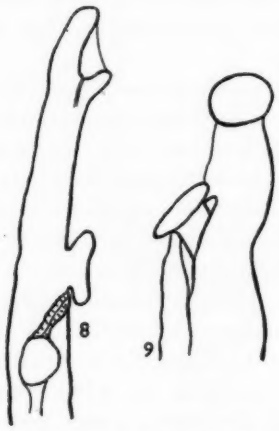
All of the female schistosomes from natural infections of *Larus novae-hollandiae* that I have examined alive and freshly collected (twenty from ten birds) have shown the oral sucker clearly. When they are left to relax, it does not matter whether they are then examined before or after fixing; the oral sucker is hard to distinguish as such.

Unfortunately, none of the original material of *Austrobilharzia terrigalensis* used by Johnston can now be found except the type slide W 443 in the Australian Museum, Sydney. On this slide are male and female worms as shown in fig. 19 in Johnston's paper; the anterior end of the female is hidden inside the gynaecophoric canal of the male, and no further details could be obtained without risking the destruction of the slide. It seems unlikely that any female schistosome is without an oral sucker, as two suckers are needed for movement against the blood stream if the female is to advance into the smaller vessels for egg-laying.

The original description of the genus *A. Johnston*, 1917, needs few alterations to fit the redescription of *A. terrigalensis* given in this paper. Points of difference are the greater range of the number of testes, the presence of the oral sucker (although the original generic description does not mention its absence in the type species), and the relative length of the body devoted to yolk follicles. The genus was redescribed and modified by Price (1929), when he added a new and

closely allied genus *Microbilharzia* with one species, *M. chapini*. *M. variglandis* was added in 1951 when Stunkard & Hinchliffe described the adult form of *Cercaria variglandis* Miller & Northup, 1926. Penner (1953*a*) concluded that *Microbilharzia chapini* was a synonym of *M. variglandis* and that the correct identification was *Austrobilharzia variglandis*; if this is so the genus *Microbilharzia* has ceased to exist.

Penner (1953*b*, 1955) is engaged on the revision of *Austrobilharzia* and allied genera.



Figs. 8, 9. *Austrobilharzia terrigalensis*. 8. Lateral view of living female worm 2 hr. after death of host, to show oral sucker. 9. Latero-ventral view of one male which shows extension of gynaecophoric canal around stalk of ventral sucker. (See text.)

SUMMARY

1. The life cycle of *Austrobilharzia terrigalensis* Johnston, 1917 is described. The larval stages occur in *Pyrazus australis*, a common mollusc on sand flats of estuaries and coastal lagoons in New South Wales.

2. *Austrobilharzia terrigalensis* was originally described from the seagull, *Larus novae-hollandiae*. Experimental infections have been successful in young gulls of this species and in the budgerigar *Melopsittacus undulatus* and the pigeon *Columba livia*.

3. Under experimental conditions the cercariae will cause dermatitis, and cases of dermatitis resembling cercarial dermatitis have been reported after bathing in Narrabeen Lagoon, where about 4 % of *Pyrazus australis* carry forktail cercariae.

4. The cercaria was described previously (Bearup, 1955) as *Cercaria variglandis* subspecies *pyrazi*, new subspecies. Now that the adult form has been identified as a previously described species the subspecific name *pyrazi* lapses.

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(*MS. received for publication* 21. IX. 1955.—Ed.)

CRAYFISH EPIDEMICS

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In 1911, Calman stated that, within the last thirty years, crayfish fisheries of Western Europe had suffered heavily from an epidemic disease, and that in this country it was responsible for the almost complete disappearance of crayfish from certain regions in the neighbourhood of Oxford. This statement apparently encouraged the scientific staff of the Bureau of Pest Control in Honolulu to inquire whether it would be possible for us to send them any of the causative organisms to try to exterminate certain of their crayfish accused of doing much harm to their crop taro (*Colocasia esculenta*).

At Professor Hardy's request I started in the spring of 1953 to study any crayfish to be found locally, as it seemed strange and remiss that up to the present no one had followed up Calman's statement as to Protozoa or other parasites being the cause of the local epidemics.

In the immediate neighbourhood of Oxford there were very few crayfish to be found at all, and none of these had any known disease. Duffield (1933, 1935), who studied the three rivers, Ock, Thame and Windrush, for fluctuations in numbers and has given much valuable information, kindly told me (personal communication May 1953) that he would let me know if any more information came his way about the return of large numbers. I have not been able to examine any specimens from the Windrush or Ock, and only one from the Ruskin Reserve, connected with the latter; this, a large male, was dead and decomposed but without known parasites. Further afield, some half dozen or so from Leney at Nailsworth in Gloucestershire were not infected, and I understand he could supply no crayfish from Haslemere. For a note on the River Thame, (see page 482).

Another river running through Oxford and not mentioned by Duffield in either of his papers is the Cherwell. I am grateful to Sir Alexander Carr-Saunders for telling me that, prior to this century, the upper Cherwell was, he understood, the place where numbers of crayfish were collected for this Department of Zoology. However, about 1904, they were no longer to be found and, when he was living at Water Eaton on the Cherwell (1929–37) they never saw or heard of a crayfish, in spite of inquiries made, and I have not been able to obtain any now.

After so many disappointments, I was very grateful to Dr Bruce Campbell for supplying us with some hundreds of crayfish collected from streams in the Wootton area, some miles north of Oxford. Among them were the only infected specimens that I had been able to investigate. and the three infecting parasites proved to be very similar to, if not identical with, those described as causing diseases in *Gammarus pulex*, the fresh-water shrimp of various regions in England (Pixell Goodrich, 1928).

The organism mentioned by Calman as a Myxosporidian was easily recognized

as the Microsporidian *Thelohania*, probably *T. contejeani* described by Henneguy & Thélohan (1892) as being the cause of an epidemic in French crayfish.

Thelohania, a genus characterized by the pansporoblast containing eight spores, is a common parasite of invertebrates. Kudo describes some thirty-three species and *T. contejeani* may be the same as *T. mülleri* described by Stempell (1902) and Léger & Hesse (1917) in the fresh-water shrimp, *Gammarus pulex*. The spores in *Astacus* appear slightly smaller, generally only $2 \times 4 \mu$, but size is of little significance, and most of these so-called species of *Thelohania* require much investigation, including early stages and the possibility of transference from one host to another.

It is well known that there is considerable difficulty in persuading Neosporidial spores to open and so liberate the infecting organism, as I found for Microsporidia in *Gammarus* in 1928, and Haplosporidia in *Chiton* (1915), and I have not been able to induce crayfish spores to open by drying and crushing or treating with gastric fluid, blood, etc. There is little doubt that it is the early merozoite stages that pass on the disease, probably directly into the blood stream by injuries inflicted during moulting, and these early merozoite stages I have not been able to obtain in *Astacus*. In the crab I have known them to prevent coagulation of the blood and so bring about early death. The spore stages in the muscles are very easy to recognize in the living crayfish by the opacity which can be seen through the cuticle of the sterna and appendages. Even the muscles of the heart show opaque streaks in badly infected specimens.

Out of 106 specimens examined from the Wootton region, nineteen were found to be infected with *Thelohania*, seven others had a microsporidian with single spores, apparently that common to *Gammarus pulex*, viz. *Nosema* ? sp., which was even more destructive to the *Astacus* muscles than *Thelohania*.

The third parasite found in ten of these crayfish from Wootton, sometimes together with one of the microsporidia, was a yeast-like organism apparently identical with that in *Gammarus pulex*, which I came to the conclusion was *Cryptococcus gammari* Vejd. In this paper (Pixell Goodrich, 1928) will be found descriptions and drawings of these parasites, and it hardly seems necessary to figure them again here—the chief necessity is to find early stages of the diseases, which are likely to be the lethal ones, and to destroy crayfish so infected before they can pass on the diseases.

There are numerous streams in the Wootton neighbourhood, and it is obvious from the percentage of infected specimens in different collections Dr Bruce Campbell kindly brought us from time to time, that crayfish from some of these streams were more infected than from others. Consequently, I am publishing this short note in the hope that someone may investigate the problem fully before all our crayfish disappear.

We were told by Dr Alan Thistle that the crayfish, native to Hawaii, has been identified by Professor Horton Hobbs, of the University of Virginia, as *Procambarus clarkii* Girard, but it is possible they also have *Astacus nigrescens*.

There is only one British crayfish and that, I am told by Charles Elton and also by J. P. Harding, of the British Museum of Natural History, is correctly named *Astacus pallipes* Lereboullet. Of course, it is by no means certain that the parasites

from our species would attack either of the Hawaiian ones, but, since our species is apparently attacked by the three parasites which prove lethal to our fresh-water shrimp *Gammarus pulex*, it seemed highly dangerous to introduce such parasites into a new locality. This danger seemed specially great since we were told by Dr A. Thistle that in Hawaii there are two families of fresh-water shrimps (Atyidae and Palaemondae) much prized for Hawaiian feasts. However, Dr Thistle wrote that such tests as were necessary could be conducted there in Honolulu.

Consequently, on 12 August 1954, I sent by air mail two dead specimens of *A. pallipes* with their muscles crowded with *Thelohania* spores and one of them marked as having the yeast in its blood as well. Immediately on death they were kept in a refrigerator till they could be packed in a chilled thermos flask for sending by air mail. I understand they arrived on 17 August, but owing to misunderstanding about their pathogenicity to human beings, both specimens were incinerated on arrival by the Hawaiian Advisory Committee, who did not consider that they had in the Islands personnel qualified to handle such organisms.

Knowing how difficult it is to get Microsporidian spores to open and so infect even their own host species, as mentioned above, I have not tried sending them, as requested, any more crayfish with *Thelohania* spores in their muscles. There is little doubt that the yeast *Cryptococcus* is more likely to be rapidly lethal to adult *Astacus* as it was found to be to *Gammarus pulex*.

With regard to the River Thame, Duffield (1933, p. 188) gave some information, saying that the river was restocked after their crayfish disappeared in 1918. In a further note (1935, p. 396) he says that they were plentiful from 1928 to 1934 but scarce again in 1935. I have no information about numbers there till 1955, when in September Mr Thrussell caught in a stream near Towersay, Thame, 300 in an hour. He kindly let us have some of these and I found that, from about 26 small specimens, three were infected with Microsporidia—one male and one female with *Thelohania* and one female with *Nosema*.

Many other parasites have been reported from crayfish in Germany, Russia and France. André & Lamy (1935) state that Dubois (1911) mentioned that Léger (1905) described a yeast in the blood which he named *Rhabdomyces duboisi*. I have, unfortunately, been unable to trace either of these papers. (Titles not given.)

Hofer (1906) incriminates a bacterium, *Bacillus pestis astaci*, as causing a disease in German *Astacus*. I have found no sign of bacterial infection except in moribund crayfish.

There is, too, the possibility of loss of crayfish by predators. Trout and other fish are sometimes found with telsons or other parts in their alimentary canals; also, no doubt, human predators account at times, especially during their breeding season, for the destruction of hundreds, as mentioned above, for Thame.

SUMMARY

Epidemics in English crayfish, *Astacus pallipes*, were found to be due to microsporidia and yeasts similar to, if not identical with, those causing diseases in another Crustacean, namely, *Gammarus pulex*, in many parts of England.

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INDEX OF AUTHORS

ARTHUR, DON R. The morphology of the British Prostriata, with particular reference to *Ixodes hexagonus* Leach. III. (With 69 Figures in the Text), 261

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. I. Incidence in some birds of Hertfordshire. (With 2 Figures in the Text), 308

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. II. Transmission by *Ornithomyia avicularia* L. (With 1 Figure in the Text), 321

BAKER, J. R. Studies on *Trypanosoma avium* Danilewsky 1885. III. Life cycle in vertebrate and invertebrate hosts. (With 5 Figures in the Text), 335

BEARUP, A. J. Life cycle of *Austrobilharzia terrigalensis* Johnston, 1917. (With 9 Figures in the Text), 470

BISHOP, ANN. Absence of potentiation between quinine and pyrimethamine in infections of *Plasmodium gallinaceum* in chicks, 220

BISHOP, ANN, HARTREE, E. F. and MCCONNACHIE, ELSPETH W. A method for determining the pH of small quantities of fluid. (With 1 Figure in the Text), 206

BISHOP, ANN and MCCONNACHIE, ELSPETH W. A study of the factors affecting the emergence of the gametocytes of *Plasmodium gallinaceum* from the erythrocytes and the exflagellation of the male gametocytes, 192

CABLE, R. M. *Opistholebes diodontis* n.sp., its development in the final host, the affinities of some amphistomatous trematodes from marine fishes and the allocreadioid problem. (With 8 Figures in the Text), 1

CHANNON, C. E. *See* HARVEY, L. A.

FERNANDO, C. H. *See* KULASIRI, C.

GIBBS, ALFRED J. *Perezia* sp. (fam. Nosematidae) parasitic in the fat-body of *Gonocephalum arenarium* (Coleoptera: Tenebrionidae). (With 15 Figures in the Text), 48

GOODRICH, HELEN PIXELL. Crayfish epidemics, 480

HARTREE, E. F. *See* BISHOP, ANN

HARVEY, L. A. and CHANNON, C. E. On *Corrigia* (*Orthorchis*) *vitta* (Duj. 1845). (With 1 Figure in the Text), 101

HOARE, CECIL A. Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *Trypanosoma evansi*. With a statistical analysis by J. W. TREVAN. (With 10 Figures in the Text), 130

HORTON-SMITH, C. and LONG, P. L. Studies in histomoniasis. I. The infection of chickens (*Gallus gallus*) with histomonad suspensions, 79

HOYTE, H. M. D. *Grahamella* (Rickettsiales) in the common shrew *Sorex araneus*. (With 1 Figure in the Text), 224

JOHRI, L. N. and SMYTH, J. D. A histochemical approach to the study of helminth morphology. (With Plate II and 1 Figure in the Text), 107

KIRTISINGHE, P. Parasitic copepods of fish from Ceylon. IV. (With 14 Figures in the Text), 14

KULASIRI, C. and FERNANDO, C. H. Camallanidae parasitic in some Ceylon fish, 420

LONG, P. L. *See* HORTON-SMITH, C.

MCCONNACHIE, ELSPETH W. Modification and elimination of the bacterial flora in cultures of *Entamoeba invadens* Rodhain, 1934, 117

MCCONNACHIE, ELSPETH W. *See* BISHOP, ANN

MEGAHED, M. M. Anatomy and histology of the alimentary tract of the female of the biting midge *Culicoides nubeculosus* Meigen (Diptera: Heleidae = Ceratopogonidae). (With 18 Figures in the Text), 22

NAJIM, ABBAS T. Life history of *Gigantobilharzia huronensis* Najim, 1950. A dermatitis-producing bird blood-fluke (Trematoda-Schistosomatidae). (With 19 Figures in the Text), 443

NEAL, R. A. Strain variation in *Entamoeba histolytica*. III. The influence of the bacterial flora on virulence to rats, 183

NEAL, R. A. and VINCENT, PATRICIA. Strain variation in *Entamoeba histolytica*. II. The effect of serial liver passage on the virulence, 173

RAUSCH, ROBERT and SCHILLER, EVERETT L. Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of *Echinococcus sibiricensis* Rausch & Schiller, 1954, on St Lawrence Island. (With Plates VII and VIII, and 3 Figures in the Text), 395

REES, GWENDOLEN. The scolex of *Tetrabothrius affinis* (Lönnberg), a cestode from *Balaenoptera musculus* L., the blue whale. (With 24 Figures in the Text), 425

RIVERS, C. F. *See* SMITH, KENNETH M.

SCHILLER, EVERETT, L. *See* RAUSCH, ROBERT

SINHA, P. K. and SRIVASTAVA, H. D. Studies on *Schistosoma incognitum* Chandler, 1926. On the synonomy and morphology of the blood-fluke. (With 5 Figures in the Text), 91

SMITH, KENNETH M. and RIVERS, C. F. Some viruses affecting insects of economic importance. (With Plates III–VI), 235

SMYTH, J. D. *See* JOHRI, L. N.

SPRENT. J. F. A. The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. (With Plate I and 29 Figures in the Text), 54

SRIVASTAVA, H. D. *See* SINHA, P. K.

THEODOR, OSKAR. On the genus *Tripselia* and the group of *Basilia bathybothyra* (Nycteribiidae, Diptera). (With 57 Figures in the Text), 353

VINCENT, PATRICIA. *See* NEAL, R. A.

WALKER, JANE B. *Rhipicephalus pravus* Dönitz, 1910. (With 11 Figures in the Text), 243

INDEX OF SUBJECTS

Absence of potentiation between quinine and pyrimethamine in infections of *Plasmodium gallinaceum* in chicks, 220
Anatomy and histology of the alimentary tract of the female of the biting midge *Culicoides nubeculosus* Meigen (Diptera: Heleidae = Ceratopogonidae), 22
Austrobilharzia terrigalensis Johnston, 1917, life cycle of, 470

Balaenoptera musculus L., the blue whale, the scolex of *Tetrabothrius affinis* (Lönnberg), a cestode from, 425
Basilia bathybothyra (Nycteribiidae, Diptera), on the genus *Tripselia* and the group of, 353

Camallanidae parasitic in some Ceylon fish, 420
Ceylon fish, Camallanidae parasitic in some, 420
Corrigia (*Orthorchis*) *vitta* (Duj. 1845), on, 101
Crayfish epidemics, 480
Culicoides nubeculosus Meigen (Diptera: Heleidae = Ceratopogonidae), anatomy and histology of the alimentary tract of the female of the biting midge, 22

Echinococcus sibiricensis Rausch and Schiller, 1954. Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of, on St Lawrence Island, 395
Entamoeba histolytica, strain variation in. II. The effect of serial liver passage on the virulence, 173
Entamoeba histolytica, strain variation in. III. The influence of the bacterial flora on virulence to rats, 183
Entamoeba invadens Rodhain, 1934, modification and elimination of the bacterial flora in cultures of, 117

Fish from Ceylon, parasitic copepods of. IV, 14

Gigantobilharzia huronensis Najim, 1950, life history of. A dermatitis-producing bird blood-fluke (Trematoda-Schistosomatidae), 443
Gonocephalum arenarium (Coleoptera: Tenebrionidae), *Perezia* sp. (fam. Nosematidae) parasitic in the fat-body of, 48
Grahamella (Rickettsiales) in the common shrew *Sorex araneus*, 224

Helminth morphology, a histochemical approach to the study of, 107
Histochemical approach to the study of helminth morphology, 107
Histomoniasis, studies in. I. The infection of chickens (*Gallus gallus*) with histomonad suspensions, 79

Ixodes hexagonus Leach. III. Morphology of the British Prostriata with particular reference to, 261

Modification and elimination of the bacterial flora in cultures of *Entamoeba invadens* Rodhain, 1934, 117
Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *Trypanosoma evansi*. With a statistical analysis by J. W. Trevan, 130
Morphology of the British Prostriata, with particular reference to *Ixodes hexagonus* Leach. III, 261

Opistholebes diodontis n.sp., its development in the final host, the affinities of some amphistomatous trematodes from marine fishes and the allocreadioid problem, 1

Parasitic copepods of fish from Ceylon. IV, 14
Perezia sp. (fam. Nosematidae) parasitic in the fat-body of *Gonocephalum arenarium* (Coleoptera: Tenebrionidae), 48

pH of small quantities of fluid, a method for determining the, 216
Plasmodium gallinaceum, a study of the factors affecting the emergence of the gametocytes of, from the erythrocytes and the exflagellation of the male gametocytes, 192
Plasmodium gallinaceum in chicks, absence of potentiation between quinine and pyrimethamine in infections of, 220

Rhipicephalus pravus Dönitz, 1910, 243

Schistosoma incognitum Chandler, 1926, studies on. I. On the synonymy and morphology of the blood-fluke, 91
Sorex araneus, the common shrew, *Grahamella* (Rickettsiales) in, 224
Strain variation in *Entamoeba histolytica*. II. The effect of serial liver passage on the virulence, 173
Strain variation in *Entamoeba histolytica*. III. The influence of the bacterial flora on virulence to rats, 183
Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of *Echinococcus sibiricensis* Rausch and Schiller, 1954, on St Lawrence Island, 395
Study of the factors affecting the emergence of the gametocytes of *Plasmodium gallinaceum* from the erythrocytes and the exflagellation of the male gametocytes, 192

Tetrabothrius affinis (Lönnberg), the scolex of, a cestode from *Balaenoptera musculus* L., the blue whale, 425
Toxocara cati (Schrank 1788), the life-history and development of, in the domestic cat, 54
Tripselia, on the genus, and the group of *Basilia bathybothyra* (Nycteribiidae, Diptera), 353
Trypanosoma avium Danilewsky 1885, studies on. I. Incidence in some birds of Hertfordshire, 308
Trypanosoma avium Danilewsky 1885, studies on. II. Transmission by *Ornithomyia avicularia* L., 321
Trypanosoma avium Danilewsky 1885, studies on. III. Life-cycle in vertebrate and invertebrate hosts, 335
Trypanosoma evansi, revision of, morphological and taxonomic studies on mammalian trypanosomes. VIII. With a statistical analysis by J. W. Trevan, 130

Viruses, some, affecting insects of economic importance, 235

The Journal of General Microbiology

Editors: B. C. J. G. KNIGHT and A. F. B. STANDFAST

Contents Volume 15, No. 3, December 1956

HALE, C. M. F. and BISSET, K. A. A comparison of the staining reactions of the cell walls of *Azotobacter chroococcum* and those of Gram-positive and Gram-negative bacteria.

PINE, L. and HOWELL, A. JUN. Comparison of physiological and biochemical characters of *Actinomyces* spp. with *Lactobacillus bifidus*.

GIBSON, F., MCDOUGALL, B., JONES, M. J. and TELTSCHER, H. The action of antibiotics on indole synthesis by cell suspensions of *Escherichia coli*.

WALT, J. P. VAN DER and TSCHEUSCHNER, I. T. *Pichia vanriji* n.sp. isolated from soil.

HENIS, Y. The adaptive formation of levansucrase by a species of Corynebacterium.

NICHOLAS, D. J. D. Trace metal requirements and some enzyme systems in a riboflavin requiring mutant of *Neurospora crassa*.

AARONSON, S. A biochemical-taxonomic study of a marine micrococcus *Gaffkya homari*, and a terrestrial counterpart.

MACLENNAN, A. P. The production of capsules, hyaluronic acid and hyaluronidase by 26 strains of group C streptococci.

POWELL, E. O. Growth rate and generation time of bacteria, with special reference to continuous culture.

MITCHELL, P. and MOYLE, J. Liberation and osmotic properties of the protoplasts of *Micrococcus lysodeikticus* and *Sarcina lutea*.

MILLIS, N. F. A study of the cider-sickness bacillus—A new variety of *Zymomonas anaerobia*.

BARNETT, J. A., INGRAM, M. and SWAIN, T. The use of β-glucosides in classifying yeasts.

WILLIAMS SMITH, H. and CRABB, W. E. The typing of *Escherichia coli* by bacteriophage: its application in the study of the *E. coli* population of the intestinal tract of healthy calves and of calves suffering from white scours.

STOCKER, B. A. D. Abortive transduction of motility in Salmonella; a non-replicated gene transmitted through many generations to a single descendant.

SERMONTI, G. Complementary genes which affect penicillin yields.

SERMONTI, G. and SPADA-SERMONTI, I. Gene recombination in *Streptomyces coelicolor*.

WAGTENDONK, W. J. VAN, TIJN, B., LITMAN, R., REISNER, A. and YOUNG, M. L. The surface antigens of *Paramecium aurelia*.

KASSANIS, B. Serological relationship between potato paracrinkle virus, potato virus S and carnation latent virus.

MORRIS, E. J. A selective medium for *Brucella* spp.

STOKER, M. G. P., SMITH, K. M. and FISET, P. Internal structure of *Rickettsia burneti* as shown by electron microscopy of thin sections.

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